# Stand Development Effects on $N_2$-fixation and Seedling Performance in Northern Boreal Forests

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Cover: Impression of the clear-cut chronosequence near Arvidsjaur
(photo: Babs Stuiver)

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## Abstract

For large parts of the Swedish boreal forests, stand development starts with clear-cutting. Still, much is unknown about how stand development affects ecosystem processes and how these processes are driven by changes in vegetation and soil properties that occur during stand development. In this thesis, the processes of feather moss associated $N_2$-fixation and tree seedling regeneration are examined along a post clear-cut chronosequence which consisted of 32 stands; eight in each of four development stages with mean ages of 4, 16, 34, and 123 years. Stand development had statistically significant effects on the total amount of N fixed at the stand level. The $N_2$-fixation rates initially increased after clear-cutting and peaked in the 16-year old stands at rates of 1.0 kg N $ha^{-1}$ $yr^{-1}$, after which the rates declined and remained at levels similar to those found in young clear-cut stands even when stands had reached maturity. This pattern was associated with high moss biomass and positive effects of local conditions on N fixed per kg moss mass in the 16-year old stands. The cumulative contribution of $N_2$-fixation to the total accrual of N per ha in the aboveground vegetation and humus layer during one stand rotation was about 9%.

Stand development had negative effects on the growth of *Pinus sylvestris* seedlings as a consequence of changes in the canopy. In contrast, the effect of stand development on *Picea abies* seedling growth was mainly driven by soil properties, and seedlings performed best when grown in soils originating from the 34-year old stands. Seedling mortality decreased with stand age for both *P. sylvestris* and *P. abies* seedlings, which was mainly caused by a positive effect of the canopy. The effect of several moss species on *P. sylvestris* seedling growth was further explored in a greenhouse mesocosm experiment. Seedling growth was most negatively affected by *Hylocomium splendens* and in pots with high moss biomass, likely driven by differences in moss depth related to moss species and moss biomass. This thesis shows that stand development has strong effects on $N_2$-fixation rates and the performance of *P. sylvestris* and *P. abies* seedlings in production forests. However, these effects and their drivers do not necessarily respond linearly to stand development and can differ between moss and tree species with potential long-term consequences for the functioning of boreal forests.



*Keywords:* bryophytes, chronosequence, clear-cut, forest regeneration, forest nitrogen balance, forest succession, logging, Norway spruce, Scots pine, understory vegetation



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Voor mijn broertje ;-)

*When I grow up*
*I want to be a forester*
*Run through the moss on high heels*
*That's what I'll do*

Text: Karin Dreijer Andersson. Covered by First Aid Kit.

# Contents

# List of Publications

This thesis is based on the work contained in the following papers, referred to by Roman numerals in the text:

I Stuiver, B.M., Gundale, M.J., Wardle, D.A., Nilsson, M-C., (2015). Nitrogen fixation rates associated with the feather mosses *Pleurozium schreberi* and *Hylocomium splendens* during stand development following clear-cutting. *Forest Ecology and Management* 347, 130-139.

II Stuiver, B.M., Wardle, D.A., Gundale, M.J., Nilsson, M-C. Seedling responses to changes in canopy and soil properties during stand development following clear-cutting (manuscript).

III Stuiver, B.M., Wardle, D.A., Gundale, M.J., Nilsson, M-C., (2014). The impact of moss species and biomass on the growth of *Pinus sylvestris* tree seedlings at different precipitation frequencies. *Forests* 5(8), 1931-1951.

Papers I and III are reproduced with the kind permission of the publishers.

The contribution of Babs Stuiver to the papers included in this thesis was as follows:

I Initiated and established the clear-cut chronosequence, performed all field work and most of the lab work, performed the statistical analyses, contributed to the design and data interpretation and led the writing of the article.

II Performed all field work and most of the lab work, performed the statistical analyses, contributed to the design and data interpretation and led the writing of the article.

III Performed the experiment and statistical analyses, and contributed to the experimental design and data interpretation and led the writing of the article.

# Abbreviations and definitions

| | |
|---|---|
| C | Carbon |
| CC | Recently clear-cut stage (mean age in 2012: 4 yr) |
| Chronosequence | Series of stands that share similar attributes, but differ in time since initiation, replacing time for space |
| *Deschampsia flexuosa* | Waivy hair-grass (sv: kruståtel) |
| *Dicranum spp* | Fork mosses (sv: kvastmossor) |
| *Empetrum hermaphroditum* | Crowberry (sv: kråkbär) |
| Field layer vegetation | Shrubs, grasses, herbs |
| *Hylocomium splendens* | Stairstep moss (sv: husmossa) |
| MF | Mature forest stage, reference for before logging conditions (mean age in 2012: 123 yr) |
| Moss layer | All moss species present on the forest floor |
| N | Nitrogen |
| $N_2$ | Dinitrogen (gaseous form of nitrogen) |
| $NH_4^+$ | Ammonium |
| $NO_3^-$ | Nitrate |
| P | Phosphorus |
| PCT | Pre-commercial thinning stage (mean age in 2012: 16 yr) |
| *Picea abies* | Norway spruce (sv: gran) |
| *Pinus sylvestris* | Scots pine (sv: tall) |
| *Pleurozium schreberi* | Big red feather moss (sv: väggmossa) |
| $PO_4^{3-}$ | Phosphate |
| *Polytrichum commune* | Common hair cap moss (sv: stor björnmossa) |
| Production forest | Non-protected forests with a tree productivity exceeding 1 $m^3$ $ha^{-1}$ $yr^{-1}$ |
| *Sphagnum girgensohnii* | Common green peat moss (sv: granvitmossa) |

| | |
|---|---|
| Stand development | The development of a forest stand from regeneration after disturbance, i.e., clear-cutting, to mature forest |
| Sv | In Swedish |
| T1 | First thinning stage (mean age in 2012: 34 yr) |
| Understory vegetation | Field and moss layer |
| *Vaccinium myrtillus* | Bilberry (sv: blåbär) |
| *Vaccinium vitis-idaea* | Lingonberry (sv: lingon) |

# 1 Introduction

## 1.1 Clear-cutting in the Swedish boreal forest

The boreal forest covers about one-third of the world's forested area, and large parts of the Swedish forest are within the boreal zone. Approximately 80% or 26 million ha of the forested area in Sweden is classified as productive forest land, with a productivity of more than 1 $m^3$ $ha^{-1}$ $yr^{-1}$ (Anonymous, 2013). The remainder of the Swedish forests have a lower productivity, or are protected, usually for nature conservation purposes (Anonymous, 2013). Most (but not all) of the productive forests in Sweden are actively managed for wood production and approximately 200 000 ha is subject each year to final felling. Forests in Sweden are commonly clear-cut and followed by active regeneration measures (Kuuluvainen et al., 2012, Lundmark et al., 2013). A clear-cut essentially involves the removal of all trees in a forest stand, with the exception of retention trees and snags that are required to be left according to the Swedish Forest Act and several forest certification schemes (Gustafsson and Perhans, 2010). After harvesting it is crucial to achieve successful forest regeneration to maintain long-term forest sustainability as required by the Swedish Forest Act. The main incentive for the first Swedish Forestry Act in 1903 was to secure forest regeneration, which clearly stated the importance of regeneration for the sustainable production of forest products (Jordbruksdepartementet, 2010, Lundmark et al., 2013). In Sweden, forest regeneration after clear-cutting typically involves soil preparation followed by planting of nursery grown seedlings. As a result most stands initiated after clear-cutting are monocultures of one species, typically *Pinus sylvestris* or *Picea abies*.

For the production of wood products, the clear-cut system has in most cases been very efficient and successful. However, there are increasing demands on forest management to provide services to society other than wood production, such as biodiversity maintenance, recreation, and production of berries and

game meat (Puettmann et al., 2009, Gamfeldt et al., 2013). The increased emphasis on these additional services means that forest stands may need to be managed differently to more closely resemble natural stands. As natural stands typically consist of multiple species and layers (Östlund et al., 1997), those stands differ substantially from the one species and one layered stands that develop after clear-cutting. An enhancement of natural regeneration, even in established clear-cut systems, is one process by which other ecosystem services could be promoted in managed forests. Natural regeneration could increase the number of tree species present in planted monocultures; and increasing tree species richness at the stand level can have positive effects on ecosystem services provided in these forests other than wood production (Gamfeldt et al., 2013). While we have an increasing knowledge about ecosystem processes in natural (unmanaged) forests, little is known about the effects of clear-cutting and subsequent stand development on ecosystem processes in the understory of boreal forests, especially in the mid- and long-term. Two such processes are studied in the context of this thesis, namely feather moss associated $N_2$-fixation and tree seedling performance. Since forest stands that have originated from clear-cutting are most common in the Swedish landscape, it is important to know how stand development following clear-cutting affects these processes as it could have implications for forest management and ultimately forest ecosystem services.

## 1.2 Stand development following clear-cutting

Clear-cutting initiates secondary succession in forests, due to the removal of the trees and disturbance of the understory vegetation and soil. The removal of the canopy markedly changes forest stand microclimate (Schmalholz and Hylander, 2009). Canopy removal allows more light to penetrate to the forest floor, increases soil temperatures during the summer and exposure to wind, resulting in increased evaporation and decline in forest floor humidity (Jemison, 1934, Økland et al., 2003). However, in clear-cut stands where drainage is poor, the decrease in evapotranspiration caused by the removal of the trees could cause the water table to rise and increase soil moisture (Keenan and Kimmins, 1993, Kreutzweiser et al., 2008). Additionally, clear-cutting causes disruptions to the forest floor due to heavy machinery and soil preparation (Fenton et al., 2003, Frey et al., 2003). Nevertheless, over time the canopy closes and the stand develops into a mature forest again. These impacts of clear-cutting and subsequent stand development will affect the environmental conditions in the stand, with consequences for ecosystem processes.

### 1.2.1 Changes in the tree layer

Our knowledge about how trees (usually planted) develop after clear-cutting is overall good, because trees have been the main object of interest for the forest owner and their management has been subject to legislation (Östlund et al., 1997). This interest in tree productivity has led to the development of management systems, which currently commonly involve both pre-commercial and commercial thinning (Kuuluvainen et al., 2012). When the trees have established and have reached a height of between 2-6 m, pre-commercial thinning is usually performed to reduce competition of naturally regenerated trees. The decision to thin (and thus reduce the number of trees per ha) is made when the canopy is closed and trees are experiencing too much competition from their neighbors, as revealed by decreasing crown length. As such, thinning can be repeated several times before the stand is considered ready for final felling. While much attention in management has been on the removal of trees in plantation forests, little interest has been given to the potential role of naturally regenerated trees, [but see Ackzell (1994) and Hallsby et al. (2015)], or how their performance is influenced by changes in above- and belowground properties following clear-cutting. In order to better understand the functioning of these managed forests, increased knowledge is needed about all components of these ecosystems and not just the trees, because many of these components can greatly impact on many processes in forest ecosystems (Walker and Wardle, 2014).

### 1.2.2 Changes in understory vegetation

Clear-cutting is commonly observed to have a negative effect on the cover of ericaceous shrubs (Hannerz and Hånell, 1997, Bergstedt and Milberg, 2001, Johnson et al., 2014). On the other hand some species, especially grasses (notably *Deschampsia flexuosa*), are favored by this disturbance and their cover rapidly increases after clear-cutting (Hannerz and Hånell, 1997, Bergstedt and Milberg, 2001, Hedwall et al., 2013, Tonteri et al., 2013). It is generally considered that with increasing time after clear-cutting grasses will decrease and dwarf-shrubs will increase as the forest stand develops (Palviainen et al., 2005). However less is known about the time frame over which these changes occur, since most studies only focus on the short term changes between pre- and post-harvest. In particular, few studies have considered the response of mosses and their development with time after clear-cutting.

Boreal forests are often characterized by an extensive moss layer on the forest floor (Turetsky et al., 2012), which plays an important role in the functioning of boreal forests (Lindo and Gonzalez, 2010, Turetsky et al.,

2012). Most mosses are poikilohydric, which means that their water content is strongly influenced by the surrounding environment (Callaghan et al., 1978, Proctor et al., 2007). As a consequence, mosses are overall negatively affected by clear-cutting due to the associated changes in microclimate (Hannerz and Hånell, 1997, Palviainen et al., 2005, Schmalholz and Hylander, 2011). However, their responses and time of recovery can differ among moss species, because moss species differ in their morphological and physiological characteristics including their water retention capacity (Palviainen et al., 2005, Elumeeva et al., 2011, Michel et al., 2012). As the stand develops following clear-cutting and the canopy closes, humidity in the stand and on the forest floor increases (Jemison, 1934), which is likely to have a positive effect on moss biomass and those species that are more dependent on higher humidity. The disturbance of the soil and the changes in microclimate due to clear-cutting are likely to result in a shift in species composition of the moss layer relative to mature forest (Johnson et al., 2014).

Several studies have shown that refuges, which contain species typical for mature forests, can be important sources for recolonization of moss species in clear-cut stands and recovery of the moss layer to pre-logging conditions (Frego, 1996, Schmalholz and Hylander, 2011, Johnson et al., 2014). As understory vegetation has been shown to play an important role in the boreal forest ecosystem (Nilsson and Wardle, 2005), changes in this layer are likely to impact on other ecosystem processes, such as feather moss associated $N_2$-fixation and tree seedling performance. Nonetheless, while initial effects of clear-cutting on understory vegetation have been relatively well studied, little is known about how understory vegetation shifts with stand development after clear-cutting over decadal time scales.

### 1.2.3 Changes in soil properties

Soil properties also change with clear-cutting and during subsequent stand development, partly because of changes in vegetation composition and partly because of the disturbance effect of the actual harvest event. Most studies on soil responses to logging have focused heavily on N, and driven by a widespread perception that growth is primarily limited by N in the boreal forest (Tamm, 1991). In general, effects of clear-cutting on soil mineral N levels in the first few years range from positive (Olsson et al., 1996, Rosén et al., 1996, Piirainen et al., 2007, Kreutzweiser et al., 2008) to neutral (Johnson and Curtis, 2001, Lindo and Visser, 2003, Kreutzweiser et al., 2008) to negative (Thiffault et al., 2011). Increased mineralization levels of N could however lead to decreased N availability in forest soils when mineralized N is subsequently lost due to leaching (Kreutzweiser et al., 2008). Whether clear-cutting has positive,

neutral or negative effects on mineral N concentrations and availability to vegetation is influenced by where it is actually measured (e.g., in the mineral soil, humus layer, or ground- or stream water) as well as key differences among locations in site conditions, climate, tree species, harvesting methods (e.g., whole tree vs stem only, or gap-size) and site preparation techniques. Additionally, the time after clear-cutting at which measurements were taken and the state of recovery of the understory vegetation can impact greatly on the results (Olsson et al., 1996, Piirainen et al., 2007, Jerabkova et al., 2011, Kurth et al., 2014, Pötzelsberger and Hasenauer, 2015). Changes in soil P levels are commonly reported as unchanged by clear-cutting, but increases in P levels have also been reported (Kreutzweiser et al., 2008). These increases were attributed to increased weathering of mineral soil after soil preparation or increased decomposition of slash and plant material following clear-cutting due to higher temperatures and moisture levels of the forest floor (Kreutzweiser et al., 2008). However, the effect of clear-cutting on soil P levels is also highly influenced by site specific conditions, much as has been observed for the effect of clearcutting on soil N levels.

As stands develop, litter decomposition has shown to decrease because of an increased contribution of litter from species that produce more recalcitrant litter and that are more common in mature forests, e.g. mosses and certain dwarf-shrub species (Oechel and Van Cleve, 1986, Nilsson and Wardle, 2005). Additionally, the soil cooling effect of mosses and canopy closure (Økland et al., 2003, Blok et al., 2011) should decrease microbial activity and thus decomposition rates during stand development (Kirschbaum, 1995). As a consequence of this decrease in decomposition rate, the mineral N availability in the soil should decrease. The total amount of N can increase though with stand development, but this N is present in larger organic compounds. However, it has also been shown that mosses can have a positive effect on decomposition and N release rates of plant litter through promoting moisture retention (Wardle et al., 2003, Jackson et al., 2013).

Additionally, studies have shown changes in species composition of the ectomycorrhizal fungal community (Jones et al., 2003) and decreases in microbial and fungal biomass during the first years after clear-cutting compared to before logging conditions (Bååth, 1980, Lindo and Visser, 2003). As most studies have focused on pre- and post-harvesting differences (i.e., within one decade after logging), few studies have evaluated the effects of clear-cutting on soil properties over a longer term perspective. Therefore, little can be said on the possible impact of soil properties on the ecosystem processes taking place in the understory over the time scale of full stand development.

## 1.3 Feather moss associated $N_2$-fixation

Previous work on feather moss associated $N_2$-fixation has almost exclusively been done in forest stands naturally regenerated following wildfire, and $N_2$-fixation in production forests has remained almost completely unstudied. This is despite growth of boreal forests being strongly limited by N (Tamm, 1991) and forest harvesting resulting in a net removal of N capital from the stand (Palviainen and Finér, 2012, Merilä et al., 2014). Any input of N to the stand is of importance, as it might offset these losses. Studies from natural forest chronosequences not subjected to harvesting have shown that feather moss associated biological $N_2$-fixation can act as a major source of N input into the ecosystem (DeLuca et al., 2002, Lagerström et al., 2007). The actual fixation is performed by epiphytic cyanobacteria that live on the shoots and leaves of the mosses in a symbiotic association; this association occurs for two of the most common feather mosses in the boreal forest, *Pleurozium schreberi* and *Hylocomium splendens* (Bay et al., 2013). The atmospheric dinitrogen ($N_2$) that the cyanobacteria fix acts as a source of new N to the forest ecosystem. The amount of N fixed by the cyanobacteria has been suggested to be driven by the N requirements of the feather moss hosts (Bay et al., 2013). The role of N limitation for $N_2$-fixation rates is also supported by field observations that show an increase in $N_2$-fixation rates as N availability declines with increasing stand age (Zackrisson et al., 2004, Lagerström et al., 2007, DeLuca et al., 2008). Other factors known to impact on $N_2$-fixation rates by cyanobacteria associated with feather mosses include moisture (Gundale et al., 2009, Jackson et al., 2011), light availability (Gundale et al., 2012a), and temperature (Gentili et al., 2005, Markham, 2009), as well as the presence of ericaceous dwarf shrubs (Gundale et al., 2010). All of these factors are expected to be impacted by clear-cutting and subsequent stand development. However, the process of this associated $N_2$-fixation has to my knowledge only been investigated in natural forests, and there is a lack of understanding about associative $N_2$-fixation in productive managed forests. It is also unknown how stand development after clear-cutting might impact on $N_2$-fixation rates. Therefore, the potential contribution of feather moss associated $N_2$-fixation to the N balance of productive managed forests, including those originating from clear-cutting, is unknown.

## 1.4 Seedling performance

Whether or not a seedling establishes and develops into a tree depends on multiple factors both aboveground and belowground. For successful establishment of seedlings at a new location, seedlings need unoccupied spaces

and access to resources, including light, nutrients and water (Craine and Dybzinski, 2013). Access to resources can be influenced by interactions with surrounding vegetation or by soil processes that influence nutrient supply rates. Limitations by multiple resources at the same time are common (Chapin et al., 1987) and the character of the most limiting resource can change depending on the developmental stage of the forest.

### 1.4.1 Impact of vegetation on seedlings

Natural regeneration of seedlings can occur continuously at all stages of stand development as long as seeds are available. The success of seedling establishment after seeding can be impacted by the surrounding vegetation, and this impact can be positive, neutral or negative (McCluney et al., 2012). The direction and magnitude of these impacts can however change with the plant's life stage and with stand development (Callaway and Walker, 1997). Positive (i.e., facilitative) responses of seedlings occur when the presence of other plants reduces stress and/or increases access to resources (Callaway, 1995, McCluney et al., 2012). Negative (i.e., competitive) responses of seedlings occur when they are suppressed by other plants that acquire the same limiting resources (McCluney et al., 2012). Competitive effects of other plants can occur aboveground through shading, or belowground through root competition for nutrients and water. Eventually, the net effect of the surrounding vegetation for the seedling is determined by the strength of these positive and negative effects at any given time.

Even though we know a considerable amount about the ecological principles of plant coexistence through competition and facilitation, uncertainties remain about the relative importance of canopy, understory vegetation and soil properties on seedling performance in managed boreal forests and how these change across forest development stages (Wagner et al., 2011). Most studies on seedling interactions with other components of the vegetation are limited to one specific stand development phase or pre- and post-harvest comparisons, or have been performed in natural fire-prone forest ecosystems (Steijlen et al., 1995, Hörnberg et al., 1997, Jäderlund et al., 1997, Wardle et al., 2008).

One of the most important and perhaps most obvious factors likely to impact seedling performance during stand development is the decrease in light availability on the forest floor as stands get older (Lieffers and Stadt, 1994, de Chantal et al., 2003b). Since light is a crucial resource for photosynthesis, the effect of canopy closure is often expected to have negative effects for seedling performance. However, limited light availability may also suppress field layer vegetation and mosses, which can compete strongly for resources with

seedlings (Steijlen et al., 1995, Jäderlund et al., 1997, Nilsson and Örlander, 1999, Hyppönen et al., 2013). Therefore, a more closed canopy could benefit seedling performance by reducing competition with understory vegetation (Kuuluvainen et al., 1993, Lieffers et al., 1999).

The effect of mosses on tree seedlings is not fully understood. Mosses can potentially have positive effects on seedlings due to their capacity to maintain moisture in the soil (Oleskog and Sahlén, 2000b, Blok et al., 2011), reduce temperature fluctuations (Soudzilovskaia et al., 2013), enhance litter decomposition and N supply rates (Wardle et al., 2003, Jackson et al., 2011, Turetsky et al., 2012), and support $N_2$-fixing cyanobacteria (Bay et al., 2013, Berg et al., 2013). Some of these effects could potentially have positive effects on soil nutrient availability. However, negative effects of mosses on seedlings are more commonly reported. Mosses can intercept and retain nutrients before they get to the seedlings (Weber and Van Cleve, 1984, Zackrisson et al., 1998, Turetsky et al., 2012), decrease decomposition because of their soil cooling effect (Blok et al., 2011), compete for light and can overgrow seedlings (Hörnberg et al., 1997, Wheeler et al., 2011) in part because of their extended growing season relative to the seedlings (Kellomäki, 1998).

The changes in microclimate caused by clear-cutting, which involves lower humidity, more fluctuations in temperatures and greater temperature extremes could have negative consequences for seedlings. In stands with good drainage, clear-cutting may decrease water availability in the top layer of the soil for the seedlings (Økland et al., 2003), and thus increase the risk of desiccation, which is a commonly reported cause of seedling mortality (Fleming et al., 1994, Greene et al., 1999). As stands get older these effects are less likely to occur, partly because of the presence of a thick moss layer. However, soil moisture can also increase due to the strong reduction in evapotranspiration following clear-cutting, especially when drainage is poor (Kreutzweiser et al., 2008, Löfgren et al., 2009).

An additional factor that is likely to impact on water availability in boreal forests in the future is climate change. The predicted changes in climate for northern Europe include longer time periods between rain events (IPCC, 2007). These prolonged dry periods could have negative effects on mosses, decreasing their growth, leading to thinner moss layers. A decreasing moss depth could be beneficial to tree seedling performance due to higher soil temperatures and less light competition (Hörnberg et al., 1997, Soudzilovskaia et al., 2011), but it could also have negative consequences due to loss of moisture from the forest floor since a thinner moss layer has a lower insulating capacity (Nilsson et al., 1996, Wheeler et al., 2011). Changes in precipitation frequency could also influence the frequency of desiccation of the mosses. Rehydration after

desiccation has shown to cause mosses to release N (Carleton and Read, 1991, Startsev and Lieffers, 2006). However, after rehydration is completed the mosses can also quickly reabsorb this N again, as such the potential contribution of these N losses to other vegetation would need further investigation (Startsev and Lieffers, 2006). As such, climate change could potentially cause a shift in effects of mosses on seedlings and nutrient availability.

### 1.4.2 Impact of soil properties on seedlings

Light and water availability for seedlings is strongly impacted by aboveground factors (such as the presence versus absence of a canopy and understory vegetation), while availability of nutrients for seedlings is additionally driven by processes in the soil. As plant growth in the boreal forest is generally strongly limited by N (Tamm, 1991), soils with a higher mineral N supply rate have shown to support higher seedling growth (Dehlin et al., 2004). Since the majority of the studies have reported elevated levels of mineral N after clear-cutting, seedlings can be expected to perform best shortly after clear-cutting (Olsson et al., 1996, Rosén et al., 1996).

In contrast, clear-cutting is often observed to exert negative effects on the soil microbial biomass and to cause changes in the mycorrhizal community (Jones et al., 2003, Lindo and Visser, 2003). This could have negative effects for seedling performance at young clear-cut sites, since mycorrhizal fungi are often positively linked to the access of plants to nutrients (including both N and P) in the boreal forest (van der Heijden et al., 1998, Van Der Heijden et al., 2008). It has been shown through a soil transfer experiment that soils from established plantations had positive effects on seedling growth, which was explained by that these established plantation had a mycorrhizal community which was better adapted to the tree seedlings (Amaranthus and Perry, 1987). Given that N and P are very important for plant growth and are expected to change over time with forest development, research is needed to understand how changes in soil nutrients impact seedling performance independently of aboveground factors that are also known to change with forest development.

## 1.5 Objectives

The overall aim of this thesis is to examine the effect of stand development following clear-cutting on feather moss associated $N_2$-fixation and tree seedling survival and growth (Figure 1). Of specific interest is the ecological role of feather mosses on ecosystem N input through biological $N_2$-fixation and on seedling performance. Specifically for this thesis I established a chronosequence with 32 stands varying in their time after clear-cutting. Along the chronosequence I investigated changes of stand characteristics and vegetation composition in order to evaluate possible drivers of specific $N_2$-fixation and seedling responses to stand development, as studied in papers I and II.

Virtually nothing is known about the role of feather moss associated $N_2$-fixation in production forests at different developmental stages. Paper I therefore investigates how $N_2$-fixation rates are affected by different development stages along the chronosequence and the potential importance of $N_2$-fixation for the total N budget of production forests. Paper II examines the direct and indirect effects of above- and belowground changes with stand development on tree seedling survival and growth of two tree species with different life history strategies. As mosses are a dominant feature of the boreal forest floor, their impact on seedling growth was studied in further detail in a greenhouse mesocosm experiment (Paper III). This paper explores the specific role of four different moss species on seedling growth at varying levels of moss biomass and precipitation frequency.

The main questions of each of the papers in this thesis are:

I During stand development following clear-cutting, how do feather moss-associated $N_2$-fixation rates change, and what factors control these changes in fixation rates?

II How does the impact of canopy, vegetation composition and soil characteristics on seedling survival and growth change during stand development following clear-cutting?

III How do different moss species common in the boreal zone affect *P. sylvestris* seedling growth, and are these effects dependent upon moss biomass and/or varying frequency of precipitation?

By addressing these questions, this thesis contributes to a better ecological understanding of boreal production forests in northern boreal Sweden and could as such contribute to improved forest management decisions in the future.

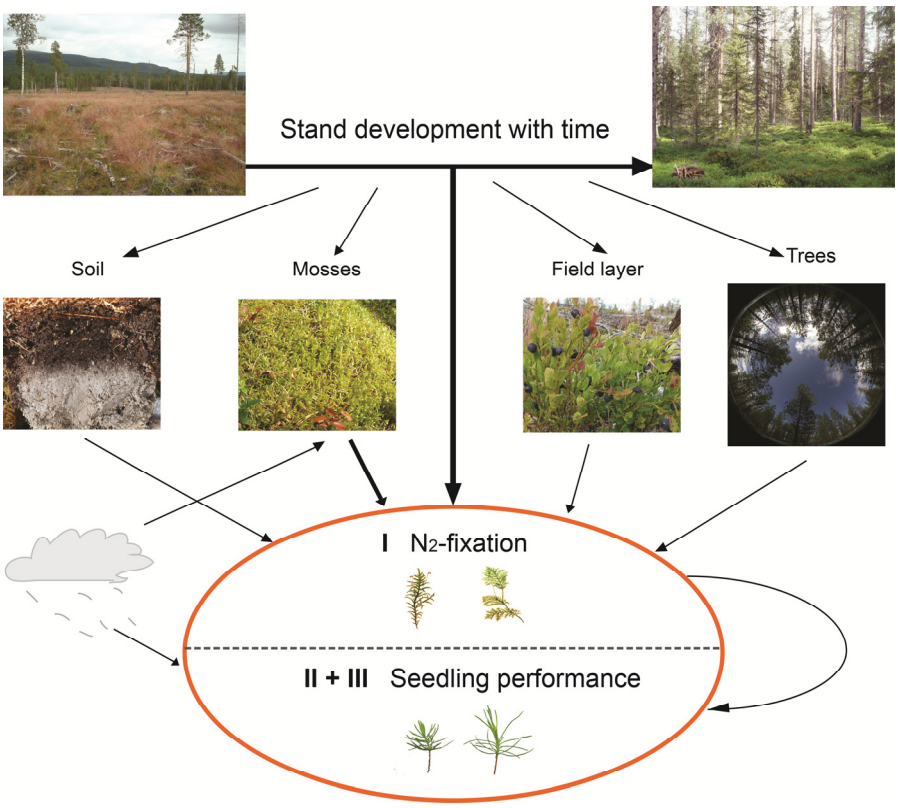


*Figure 1.* Conceptual framework of the potential effects of stand development on the properties of soils and vegetation (i.e., mosses, shrubs and trees) that can in turn affect seedling performance and $N_2$-fixation rates. Roman numbers refer to papers I, II and III. Moss drawings: www.ecosystema.ru. Photos: Babs Stuiver.

# 2 Materials and Methods

## 2.1 Study system

### 2.1.1 Clear-cut chronosequence

The impact of time on processes in the forest is difficult to study, as forests grow slowly and exceed the career-span of individual researchers. Therefore, the use of chronosequences is an alternative approach that allows to study the effect of time when succession follows a predictable trajectory, such as succession after wild-fires or clear-cutting (Walker et al., 2010). A chronosequence substitutes time for space, and thus allows simultaneous comparison of different successional stages.

In paper I and II, the effect of time (i.e., stand development) on feather moss associated $N_2$-fixation rates and seedling performance was studied along a chronosequence consisting of 32 stands. This chronosequence was identified in 2012 and situated in the surroundings of the town Arvidsjaur (65°35′N-19°10′E) in northern Sweden (Figure 2). The average annual temperature and precipitation in this area were 1.2 °C and 542 mm, respectively, over the period 2002-2013 (Swedish Meteorological and Hydrological Institute, 2014). Stands were selected in such a way that they all shared the same site productivity index, vegetation type [*Vaccinium myrtillus* (Hägglund and Lundmark, 1999)] and sandy silt glacial soil type.

The 32 stands were divided into four stand development stages (Figures 3 and 4), which were of the following types and mean ages in 2012, with 8 replicate stands for each development stage:

- CC: clear-cut and recently planted with containerized *Pinus sylvestris* seedlings, 4 years old
- PCT: pre-commercial thinning stands, 16 years old
- T1: first commercial thinning stands, 34 years old
- MF: uncut mature forest that will typically be clear-cut within the following five years, 123 years old

All clear-cut stands (i.e., CC, PCT and T1) underwent mechanical site preparation followed by planting of nursery grown seedlings of *P. sylvestris*, resulting in *P. sylvestris* tree monocultures. The MF stands were dominated by *P. sylvestris*, but *P. abies* was growing into the canopy from below. The classification used for the four stages also coincides with the classification system used for Swedish forest management plans.

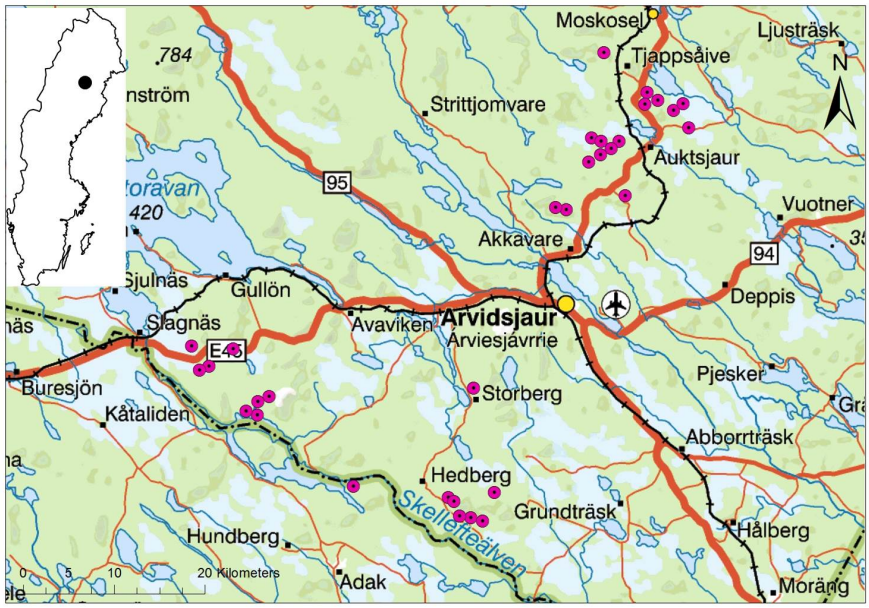


*Figure 2.* Locations of the 32 stands forming the post clear-cut chronosequence. © Lantmäteriet

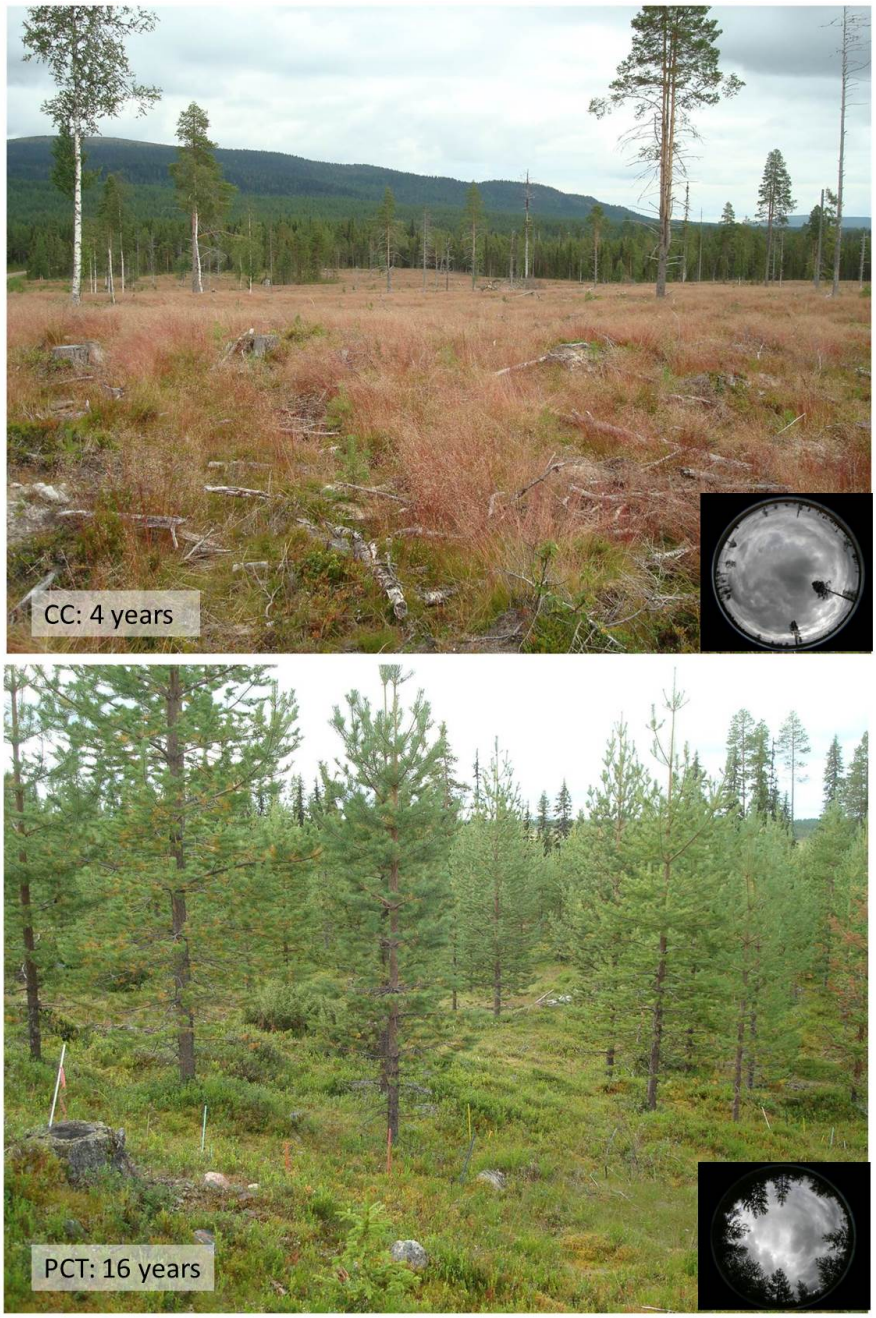


*Figure 3.* Representative image of a clear-cut stand (CC), with abundant presence of *D. flexuosa* on the forest floor, and a pre-commercial thinning stand (PCT) with *V. myrtillus, V. vitis-idaea* and *P. schreberi* in the understory. The fish-eye photograph in the lower-right corner of each panel shows the representative canopy cover in that stand type. Photos: Babs Stuiver

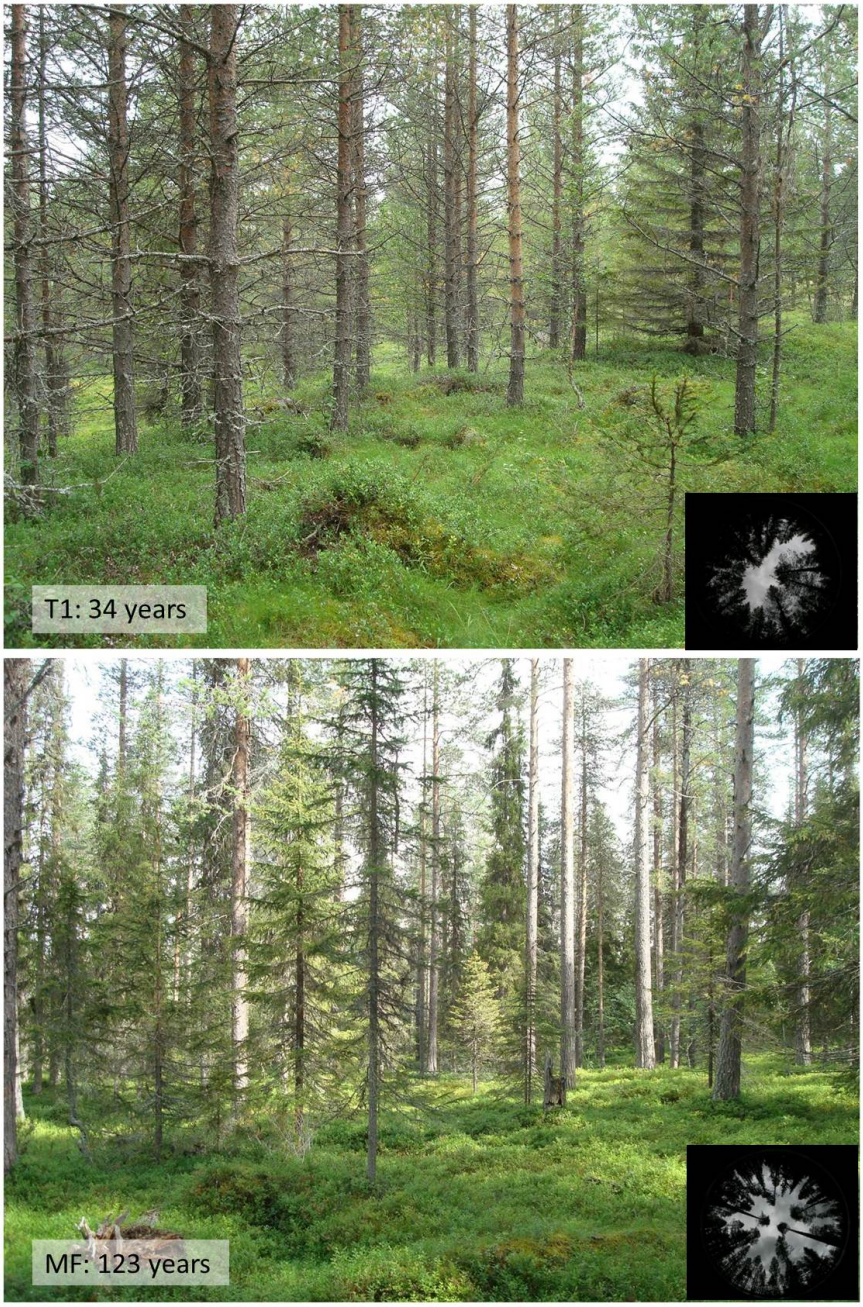


*Figure 4.* Representative image of a first thinning stand (T1), with abundant *V. myrtillus, V. vitis-idaea* and *P. schreberi* in the understory, and a mature forest stand (MF) dominated by *P. sylvestris*, but with *P. abies* growing into the canopy and *V. myrtillus* in the understory. The fish-eye photograph in the lower-right corner of each panel shows the representative canopy cover in that stand type. Photos: Babs Stuiver

### 2.1.2 Greenhouse experiment

In order to test for the specific effects of mosses and different precipitation regimes on seedling growth, a mesocosm pot experiment was set-up in a greenhouse (Paper III). During the experiment, environmental conditions were set at a temperature of 16 ± 2 °C, a relative humidity of 70 ±10% and a radiation of 120 μmol $s^{-1}$ $m^{-2}$ with an 18-hour day length; this resembles growing season conditions in northern Sweden. Dense monocultures of the moss species, *Pleurozium schreberi*, *Hylocomium splendens*, *Polytrichum commune* and *Sphagnum girgensohnii*, were collected for use in the experiment in September 2011 at three different locations across northern Sweden: Vaksliden (65°40′ N 18°53′ E), Kulbäcksliden (64°9′ N 19°35′ E), and Sävar (64°0′ N 20°34′ E). The forests from which the moss samples were taken were mixed forests of *P. sylvestris* and *P. abies*, and were at least 80 years old.

## 2.2 Study design

### 2.2.1 Feather moss associated $N_2$-fixation

For the study in paper I, I measured $N_2$-fixation rates along the chronosequence using the same procedure as described by others (Lagerström et al., 2007, Gundale et al., 2012b). This meant that 10 shoots of *P. schreberi* and 5 shoots of *H. splendens* were collected at each of ten random locations in each of the 32 stands constituting the chronosequence (i.e., CC, PCT, T1 and MF-stands, Figures 3 and 4) in August of both 2012 and 2013. Shoots were collected as close to the center point of the random location as possible to ensure that ten complete samples of each species were always collected at each stand. These shoots were then placed in 22 ml glass vials, one for each sample of each species before being transported to the laboratory for analyses (Figure 5). The vials were stored in a climate chamber at 16 °C and 20 h light-4 h dark cycle until analyses were performed.

In order to test for long-term storage effects on $N_2$-fixation rates prior to measurement, a pilot study was performed. This involved collecting an extra set of 10 vials of *P. schreberi* samples (i.e., 10 shoots per vial) in each of 11 selected stands in August 2013 [i.e., CC (n=2), PCT (n=3), T1 (n=3) and MF (n=3)]. Collection of these samples was done using the same procedure as described above. Upon arrival in the lab these samples were stored in the same climate chamber as were the other samples, and analyzed at 1, 6, 8, and 60 weeks after collection in the field.

*Figure 5.* Shoots of *P. schreberi* (left) and *H. splendens* (right) in 22 ml vials upon arrival in the lab. Photo: Babs Stuiver

### 2.2.2 Transplantation experiment

To simultaneously test for the effects of aboveground (stand type) and belowground (soil origin) characteristics on tree seedling survival and growth, a full-factorial transplantation experiment was carried out (Paper II). This experiment utilized the three youngest stand development stages of the clear-cut chronosequence (i.e., CC, PCT and T1-stands, Figures 3 and 4). In short, soil from each stand was moved to one stand from each of the other two stand development stages. This was done by first assigning each stand to one of eight groups, so that each of the groups contained three stands, one of each development stage (Figure 6). In each stand, six planting locations were established each containing four soil origin classes; one removed and returned to the same location, two that were removed and replaced by the other two stand types, and one that served as a non-removed control (Figure 6). The 432 cores were extracted using a 14.8 cm diameter PVC-tube equipped with a saw-blade in July 2012 (Figure 7). Cores were 15 cm deep from the soil surface to the bottom and were moved between stands as shown in Figure 6. Before the cores were placed back in the ground, humus depth and moss depth was measured.

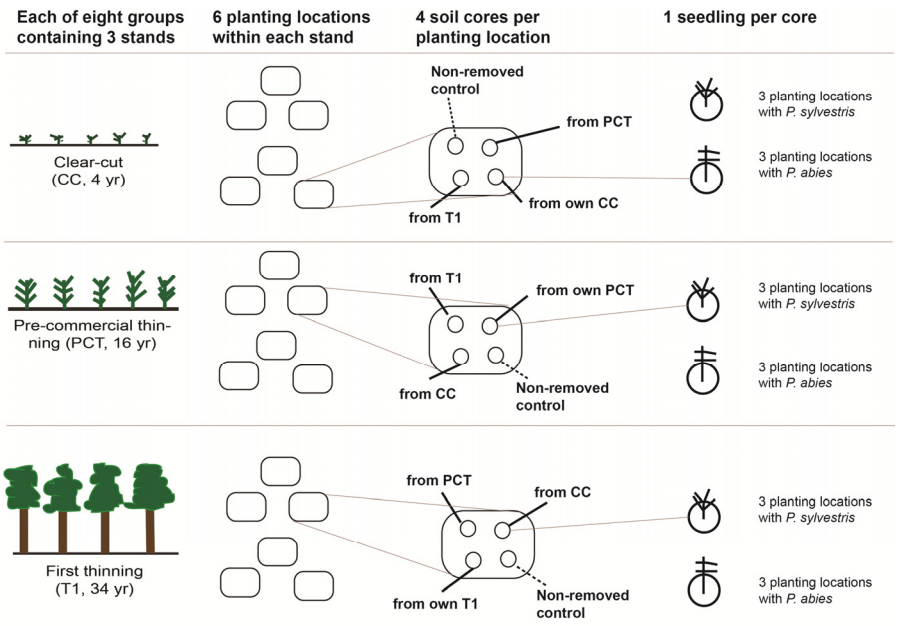


*Figure 6.* Schematic overview of the transplantation experiment and the distribution of in total 432 cores across stands. In total there were 8 replicates of each group containing one stand of each of three developmental stages.

Two weeks after the transplantation of the cores was executed, half of the planting locations (i.e., 3 of the 6) were planted with one *P. sylvestris* seedling in each of the 4 cores and the other half were planted with one *P. abies* seedling in each of the 4 cores (Figure 6 and 7). The size of the cores was expected to be large enough to prevent roots from the seedlings growing out of the cores during the course of the experiment. These were then left to grow during three growing seasons and were destructively harvested in August 2014. Prior to harvest seedling heights were measured in the field. Seedlings were extracted from the cores after which shoots and roots were separately dried and weighed.

*Figure 7.* A core taken in early July 2012 (left), a *P. sylvestris* seedling in September 2012 (middle) and a *P. sylvestris* seedling at time of harvest in August 2014 (right). Photos: Babs Stuiver

### 2.2.3 Greenhouse mesocosm experiment

The study in Paper III was a 204-pot greenhouse mesocosm experiment, set-up in December 2011 to test for the effect of mosses and precipitation frequency on tree seedling growth. All pots contained 3 cm of quartz sand, and on top of this 5 cm of sieved humus (collected from Kulbäcksliden) was placed. The pots were arranged in 12 blocks according to a full-factorial split-plot design (Figure 8). Each block contained 17 pots that were prepared to be later planted with *P. sylvestris* seeds. One of these pots was a moss free control, while of the other 16 pots four pots were planted with each of the four moss species (i.e., *Pleurozium schreberi*, *Hylocomium splendens*, *Polytrichum commune* and *Sphagnum girgensohnii*). Additionally, moss biomass was also manipulated, which resulted in that of the 4 pots of each moss species, one was assigned to each of the following four moss biomass classes (MBC, i.e., % of the reference dry weight moss biomass value); 80% (500 g $m^{-2}$), 40% (250 g $m^{-2}$), 20% (125 g $m^{-2}$) and 8% (50 g $m^{-2}$) (Figure 8). The additional moss-free control pot represented the no moss cover (0%) MBC. The dry weight reference values were derived from dense monospecific moss cushions for each moss species collected in the field. In order to construct the pots with the accurate MBC, a relationship between fresh and dry weight was established. In order to derive the fresh weight I used a method described by Fenton et al. (2010). This method was also used to get the correct fresh weight in relation to each MBC for each species, after which the moss layer in the pots was reconstructed shoot by shoot.

To justify how the MBC values used in the mesocosm experiment related to the moss biomass values found along the clear-cut chronosequence, moss biomass values from the chronosequence were “standardized” for their cover.

This meant that mean moss mass values were calculated for the area of the stand actually covered by moss. As such, the average mass of the moss cushion a seedling would be growing in was derived for each stand along the chronosequence.

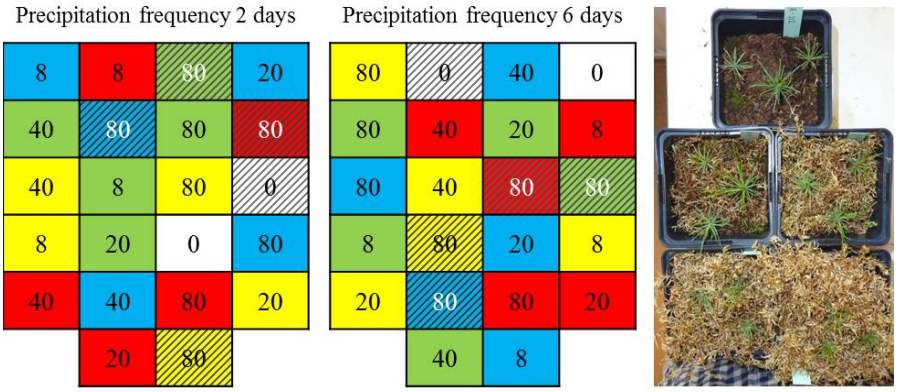


*Figure 8.* Schematic overview of the experimental lay-out of two out of 12 blocks with precipitation frequencies of watering each 2 days (left) or 6 days (middle); each square represents one pot. The different colors represent the different moss species: *P. schreberi* (green), *H. splendens* (purple), *P. commune* (orange) and *S. girgensohnii* (yellow) and the number (0, 8, 20, 40, 80) in each pot represents the percentage of moss relative to the reference samples in each of five moss biomass classes (MBC). The pots marked with 0 are the moss free pots. The striped pots do not contain seedlings, while all other pots contained three *P. sylvestris* seedlings. The photo on the right depicts *P. sylvestris* seedlings grown in pots covered with *P. schreberi* and in each of five MBC (i.e., from top to bottom and left to right 0, 8, 20, 40, 80%) at the end of the experiment. Photo: Babs Stuiver

In 17 pots in each block, ten newly germinated seeds of *P. sylvestris* were planted in each pot by carefully placing the seed on top of the humus layer, 12 days after the construction of the moss layer. After six weeks only the three most vigorous seedlings were selected and kept for the remainder of the experiment (Figure 8). At this time the precipitation frequency treatment was also initiated with half of the blocks (randomly selected) receiving ambient frequency (i.e., watering every second day), and the other half receiving a reduced frequency (i.e., watering every sixth day). All pots independent of precipitation frequency received the same total amount of deionized water, which was equivalent to a monthly precipitation of 60 mm. The ambient precipitation frequency was based on observations by the Swedish Meteorological and Hydrological Institute in the Arjeplog area between 2007 and 2010 (Jackson et al., 2011). The reduced precipitation frequency simulates the expected changes in rainfall frequency during summer induced by climate changes, with longer periods between rain events and more heavy rains when

they do occur (Schlyter et al., 2006, IPCC, 2007, May, 2008). After another 12 weeks the experiment was terminated. Moss depth was measured before the pots were destructively harvested. For each pot the dry weight of the mosses was determined, so that the moss biomass that accumulated during the experiment could be determined. The seedlings were extracted from the pots by rinsing the soil from the roots with water, and for each seedling height, shoot and root biomass was measured.

In each of the 12 blocks an additional five pots were present without seedlings. One of these pots was a moss free control, while the other four pots were covered with an 80% MBC moss layer of each moss species. These pots were used to assess possible effects of the mosses on the soil nutrient status of the pots as well as the rates of associated $N_2$-fixation for each moss species. To assess availability of mineral N and P in the soils of these pots, one resin capsule (PST1 capsule, Unibest Bozeman, MT, USA) was placed in the humus layer of each pot at the time of set-up and retrieved from the pots after 20 weeks for measurement. The sampling and preparation of the mosses for $N_2$-fixation measurements was performed according to the same procedures as described in section 2.2.1, for *P. commune* and *S. girgensohnii* 10 and 5 shoots were collected, respectively. From each pot three separate vials were prepared.

## 2.3 Sample collection of stand data

Data for a large variety of variables was collected to describe stand characteristics in each of the 32 stands along the chronosequence (Figure 9). This data was later used to test for possible drivers of possible differences in $N_2$-fixation rates and seedling performance between stand development stages (paper I and II). Within each of the 32 stands along the chronosequence a predetermined sampling area of 2500 $m^2$ was selected where all data sampling was performed.

### 2.3.1 Trees and canopy

Several characteristics of the trees were measured in each stand to describe the development of the tree layer along the chronosequence. In five 100 $m^2$ random circular plots in each stand tree diameter at breast height (i.e., at 1.3 m) and tree height were measured and used to calculate tree biomass. The average number of trees per ha was determined from all the measured trees within these circular plots. A characteristic that was expected to influence many processes in the stands and that would be highly influenced by the tree canopy was light transmission, and this was measured by using hemispherical photographs using a fish-eye lens at three random locations in each stand. Light transmission

represented the proportion of radiation under the plant canopy relative to that in the open. Litter fall for each stand was estimated by collecting litter that fell into three randomly placed trays (38 × 58 cm) in each stand over a period of 11 months (September 2013-August 2014). With this method the majority of the litter originates from the trees.

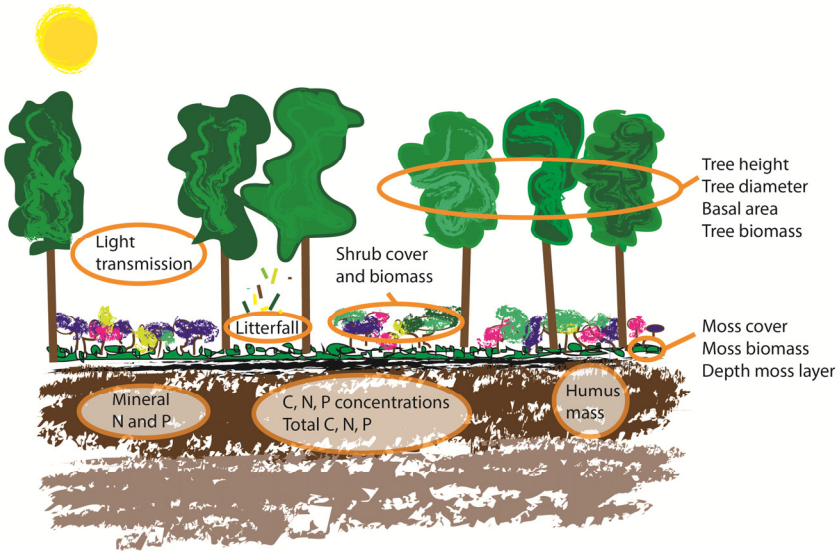


*Figure 9.* Schematic overview of the measured and estimated stand characteristics for each of 32 stands along the chronosequence.

### 2.3.2 Field and moss layer

In order to determine the species cover in the field and moss layer, 20 random 50 × 50 cm plots (Hannerz and Hånell, 1997) were established within the sampling area of each stand. In each of these plots, cover estimates for all vascular plants comprising in the field layer vegetation and all moss species in the moss layer were determined using the Braun-Blanquet method (Van Der Maarel, 1975). This method uses a classification principle with 9 classes that vary in abundance and cover.

For 10 of these twenty random plots, all vascular plant material was removed from the center 25 × 25 cm by clipping as close to the ground as possible to determine biomass of each plant species present. This sample size was smaller than for cover estimates for practical reasons, and has been used by others (Kauffman et al., 1994). Additionally, all moss material, but not moss litter, was removed on an 81.7 $cm^2$ circular area (cut out by a PVC tube with sharpened edges) from 15 of the twenty random plots for each stand to determine moss biomass. To start with ten moss samples were analyzed; later

the remaining five samples were also analyzed, which proved not to add to the variation in the stand. These 15 samples from each stand therefore give a good representation of the moss species and its biomass present in the stands across the chronosequence. Further, moss depth measurements were taken in ten of these plots.

### 2.3.3 Soil

The full depth of the humus layer in each stand was sampled using a 14.8 cm diameter PVC tube equipped with a sawblade, as used in the transplantation experiment, at ten random locations. Since a random sampling method was used, samples without humus were also included when sampling points were located on bare mineral soil; as such the sampling covered the heterogeneity of the stand. Humus depth was recorded at two perpendicular sides for each of the removed humus cores. The humus from each core was retained and then sieved (8 mm mesh), dried and weighed in order to determine the bulk density of the humus layer (i.e., g $cm^{-3}$). The average bulk density and humus depth in each stand were then used to calculate the humus mass to an areal basis. All the humus samples of each stand were then mixed to get one composite sample for each stand. Soil nitrogen (N), phosphorus (P) and carbon (C) concentrations were determined for each of these composite samples, and were later scaled-up to total pools of each element using the humus mass that was calculated for each stand. Availability of inorganic nutrients such as $NH_4^+$–N, $NO_3^-$–N and $PO_4^{3-}$–P was derived from resin capsules that were placed on the interface of the moss and humus layer during one growing season (i.e., June-September 2013).

## 2.4 Sample processing and calculations

### 2.4.1 Vegetation biomass

For each stand, total biomass of each plant species in the tree layer, ground vegetation and moss layer was determined to enable assessment of species composition and of how the individual species changed with stand development. For trees, biomass was calculated from tree height and diameter at breast height (dbh) measurements using specific Marklund's equations for each tree species (i.e., *P. sylvestris, P. abies* and *Betula* spp) (Marklund, 1988). The collected field layer vegetation clippings and removed moss samples from each stand (see above section 2.3.2) were transported to the lab, where they were sorted to species, dried at 70 °C for 48h and weighed. These values were then scaled up to an areal basis (e.g., $ha^{-1}$).

### 2.4.2 Soil nutrient analyses

All resin capsules from the chronosequence and the greenhouse experiment (paper I-III) were processed according to the same procedure. They were subjected to three separate extractions by 10 ml 1 M KCl that were shaken for 30 minutes each. The solutions were then analyzed for their $NH_4^+$, $NO_3^-$ and $PO_4^{3-}$ concentrations using an Autoanalyzer 3 (SEAL Analytical). For paper I and II total soil N, P, and C concentrations for each stand along the chronosequence were derived from each composite humus sample (i.e., one collective sample per stand), using three sub-samples that were ground in a ball-mill. Concentration of C and N were derived by combustion and analyzed with an Elemental Analyzer (Werner et al., 1999), for P samples were prepared by acid digestion (8% $H_2SO_4$) and analyzed with an Auto Analyzer. For paper I, these values were used to determine total N, P and C pools in the humus layer per unit area for each stand, using the calculated humus mass values.

### 2.4.3 $N_2$-fixation analyses

In paper I and III, $N_2$-fixation rates associated with mosses were measured using an acetylene reduction assay, as described in previous studies (Lagerström et al., 2007, Gundale et al., 2012b). In short, before analyses the moss samples in each vial (Figure 5) were fully hydrated, after which the vials were sealed and injected with acetylene. The vials were then left in a climate chamber for 24-h at 16 °C. Ethylene concentrations in each vial were measured on a gas chromatograph using ethylene standards (Clarus 500 GC, PerkinElmer Inc. Waltham, Massachusetts). These values were then converted into ethylene production, using a ratio of 3 mol of ethylene reduced per mol N fixed (DeLuca et al., 2002). Values were then expressed as mg N kg $moss^{-1}$ $d^{-1}$ and µg N g $moss^{-1}$ $d^{-1}$ for paper I and III, respectively. These mass values were derived from the moss mass of each sample (i.e., vial), which was dried and weighed after analyses. For paper I these values were further determined as values per unit area (kg N $ha^{-1}$ $yr^{-1}$) using the moss biomass measured from the stand vegetation composition data, and scaled up to annual values assuming a 200 day growing season (DeLuca et al., 2002).

The moss samples of the long-term storage pilot study were analyzed according to the same procedure as described above, but the same samples were analyzed 1, 6, 8 and 60 weeks after sampling. Between analyses the samples were maintained in their vials, kept moist and stored in a climate chamber at 16 °C with 20 hours light per day. After the 60 week analysis, all samples were dried and weighed. The dry weight of each sample was then used to determine $N_2$-fixation rates per moss mass at 60 weeks. For the calculation of the $N_2$-fixation rates per unit moss mass at 1, 6 and 8 weeks, the average vial

weights of the *P. schreberi* samples (from 2013) for each stand from the study in paper I were used. These weights were more representative than the dry weight of the samples after 60 weeks, because the mosses grew considerably during the storage period.

### 2.4.4 N pool estimations

In order to estimate the relative contribution of $N_2$-fixation to stand N balance several calculations were done (Paper I). First, for each of the 32 stands the N pools (aboveground) were determined for trees, field layer vegetation, grasses and mosses, using their measured biomass values and literature derived N concentrations. Further, the total humus N pool values were calculated as described above. Second, values of each of these N pools were then averaged across all replicates of each stand development stage (n=8). The combined pools of all vegetation and humus pools were then added to obtain a total N pool present in each stand development stage. Third, the difference in the total N pool present between MF-stands and CC-stands was calculated and this value was assumed to represent the N that had accumulated in the stand during one rotation period after clear-cutting.

Finally, the total N input from feather moss associated $N_2$-fixation was calculated for the whole rotation period. Since $N_2$-fixation rates did not show a linear relationship with time since clear-cutting, the calculation of total input from $N_2$-fixation was based on the addition of the amount of N fixed $ha^{-1}$ $yr^{-1}$ between each of the four stand development stages. The between development stage values were determined as the mean of the annual fixation rates $ha^{-1}$ of two consecutive stages multiplied by the number of years between these stages. Summing this for all development stages gave the total amount of N fixed associated with both *P. schreberi* and *H. splendens* during the rotation period. The accumulated amount of N fixed was then expressed as a percentage of the total N accrual between the CC and MF stands. This value represents the relative contribution of $N_2$-fixation to the accumulated N pool in the aboveground vegetation and humus layer during one stand rotation.

## 2.5 Statistics

For paper I and II, differences for all stand characteristics between stand developmental stages were analyzed using one-way ANOVA. The effects of stand development stage (i.e., CC, PCT, T1 and MF) on moss biomass and $N_2$-fixation rates (Paper I) were tested with two-way ANOVAs with moss species and stand development stage as fixed factors. The effects of long-term storage in the pilot study were tested with one-way repeated measures ANOVA, with

number of weeks in storage as the within subject factor and stand development stage as the between subject factor.

In order to test which variables were the main drivers of stand type differences the aboveground and soil properties were reduced into fewer variables. This was done by means of a Principal Component Analysis (PCA) on the square root-transformed data of 18 stand attributes (i.e. stand age, light transmission, pool size of soil N, P, and C, concentrations of soil $NH_4^+$, $NO_3^-$ and $PO_4^{3-}$, and biomass of trees, *V. myrtillus, V. vitis-idaea, E. hermaphroditum, D. flexuosa*, total shrubs, herbs, *P. schreberi, H. splendens*, and total moss; humus mass was not included in the analysis due to co-linearity with nutrient pool sizes).

For paper II the CC, PCT and T1 stands were used to test the effect of aboveground (stand type) and belowground (soil origin) characteristics on seedling survival at the end of each growing season. This was done using a repeated measures three-way linear mixed model. Effects of stand type and soil origin on seedling growth were tested using a three-way linear mixed model. For both mixed models stand type, soil origin and tree seedling species were used as fixed factors, and transplantation group and species nested within individual stand were used as random factors. Subsequently the effects of stand type and soil origin on the number of seedlings that survived all three growing seasons and on seedling growth were also tested for each species separately with a linear mixed model and a two-way ANOVA respectively. For each tested variable along the chronosequence the unit of replication was the number of stands per stand development stage (n=8). For all ANOVAs interactive effects were also tested for, and whenever significant effects were indicated by ANOVA analyses, Tukey's HSD post-hoc tests were then performed to identify differences between main factors (such as moss species, MBC, moss biomass, stand type or soil origin) or between combinations of main factors. Whenever significant effects for seedling survival and growth in paper II were indicated by a linear mixed model adjusted Bonferroni post-hoc tests were done to identify differences between factors. When necessary, data was transformed to meet the requirements of ANOVA and mixed model.

Spearman's Rho correlation analyses were performed to test for relationships of stand characteristics with $N_2$-fixation rates (paper I) and seedling survival and growth (paper II). For the correlation analyses each stand was treated as an individual data point. All analyses on data from the chronosequence were performed in IBM SPSS Statistics 19 (Paper I) and 22 (Paper II).

For the responses of tree seedling growth to moss species, MBC, and precipitation frequency in the greenhouse mesocosm experiment (Paper III) a

three-way analysis of variance (ANOVA) was used. The effect of moss species and precipitation frequency on $N_2$-fixation, $NH_4^+$, $NO_3^-$ and $PO_4^{3-}$ were tested using two-way ANOVA with moss species and precipitation as fixed factors for the seedling free pots. The effect of MBC on moss depth across all species as well as the effect of moss species on seedling height and moss depth in the 80% MBC pots were tested with a one-way ANOVA. In all ANOVAs block was used as a random factor. These analyses were performed in R v 2.12.1 from R Core Team (2012).

# 3 Results and Discussion

Since the 1950's the clear-cut forest management system has created the most common successional pathway for forests in Sweden (Östlund et al., 1997, Kuuluvainen et al., 2012, Lundmark et al., 2013). This system involves the removal of (almost) all the trees by large machines followed by mechanical soil preparation and planting of nursery grown seedlings. Surprisingly little is still known about what happens in the understory of these forests during or following clear-cutting, as the focus has mainly been on tree production (Östlund et al., 1997, Kuuluvainen et al., 2012). This thesis has therefore focused on two important ecological processes that take place in the forest understory of actively managed production forests in northern Sweden, namely biological $N_2$-fixation associated with feather mosses and the early growth of tree seedlings. The overall aim of this thesis was to examine the effect of stand development following clear-cutting on feather moss associated $N_2$-fixation and tree seedling performance. However, in order to evaluate possible drivers of $N_2$-fixation and tree seedling performance in the understory, this thesis first explored how stand development impacted on vegetation and soil properties. Another emphasis in this thesis was on the role of mosses, as they are highly abundant on the forest floor as is characteristic for northern boreal forests. For this work, a post clear-cutting chronosequence was established, which consisted of 32 stands that differed in time after clear-cutting and were classified in four development stages (stand types); recently clear-cut (CC), pre-commercial thinning stage (PCT), first thinning stage (T1) and mature forest (MF). The major findings and their possible implications for forest management are now discussed.

## 3.1 Impact of stand development on vegetation and soil

Forest stands take a long time to develop and chronosequences can therefore be used to study stand age effects on ecological processes (Walker et al., 2010). However, when using the chronosequence approach one has to be aware of its potential limitations. Its use can be misleading when used to infer successional replacement of species driven by time, because other factors responsible for plant community trajectories cannot be excluded (Johnson and Miyanishi, 2008). The chronosequence used in this thesis meets the most important criteria to justify the appropriate use of a chronosequence for the purpose of this thesis (Walker et al., 2010). Firstly, the stands in the clear-cut chronosequence follow a predictable trajectory from regeneration to final felling. This is further strengthened by the fact that all stands are typical examples of each of the specific management stages. Ideally, the oldest stage (MF) would also have originated from clear-cutting. Still, these MF stands are relatively even-aged and are managed for production purposes. However, as the MF stands were not established from clear-cutting, these stands were not used for the soil transplantation experiment (paper II). Secondly, all stages were properly replicated (n=8) and located across a large geographical area. All measurements within each stand have also been replicated to adjust for within-stand heterogeneity. Thirdly, this study system also has the advantage that it contains relatively few dominating species (e.g., *P. sylvestris*, *V. myrtillus*, and *P. schreberi*), which makes it likely that stands follow the same trajectory. Finally, the chronosequence approach is the best available tool to test for effects of time on ecosystem processes due to time constraints for this thesis and the lack of long-term experiments meeting the aim of this research. An important consideration is that the effect of time in these production forests cannot solely be attributed to inherent ecological processes, but also by the indirect effects of time associated with management practices. All in all, the results from this thesis should mainly be used to improve our general understanding of the development of *P. sylvestris* dominated stands after clear-cutting in northern Sweden and not as a predictive pathway for the individual stand or all *P. sylvestris* dominated stands across Europe.

### 3.1.1 Trees - canopy

Tree biomass increased as the chronosequence proceeded and stands developed; this led to increasing canopy closure, and 34 years after clear-cutting light transmission through the canopy had decreased to around 30% (Table 1 and paper I). Due to the selection criteria and the way the clear-cut stands were regenerated, the tree layer throughout the chronosequence was dominated by *P. sylvestris* as this was the planted species after stand initiation.

The mature forest stands are likely to have developed following fire and more gradual logging. As a result, *P. abies* had the opportunity to regenerate and develop into the canopy. Increasing tree biomass with stand development meant that litter input from the trees (i.e., needles and twigs) increased accordingly (Table 1 and paper II).

### 3.1.2 Field layer vegetation

Responses of field layer vegetation to clear-cutting and following stand development differed depending on species. In agreement with other studies (Hannerz and Hånell, 1997, Bergstedt and Milberg, 2001), *D. flexuosa* biomass and cover were highest in the youngest stands, i.e., shortly after clear-cutting (Table 1 and paper I). Dwarf shrub biomass was initially negatively affected by clear-cutting, but increased rapidly and reached maximum values at the PCT stands (Table 1). *Vaccinium myrtillus* was the dominant dwarf-shrub species and therefore showed a similar response. For the other main dwarf shrub species, i.e., *V. vitis-idaea* and *E. hermaphroditum*, statistically significant differences could not be identified between stand developmental stages, although they both showed the highest values in PCT and T1 stands. The relatively fast recovery of *V. myrtillus* after clear-cutting was not expected, since several studies have reported long-term decreases of *V. myrtillus* at the landscape scale, which is attributed to the increasing density of clear-cut sites in the landscape (Hedwall et al., 2013, Tonteri et al., 2013). The results in this thesis showed however that *V. myrtillus* biomass recovered within a couple of decades, in line with findings by Palviainen et al. (2005). Whereas total dwarf shrub biomass already reached the highest values in PCT stands, dwarf shrub cover recovered at a slower rate; it did not reach maximum values until the T1 stage and remained high in the mature forest (Figure 10).

Table 1. *The overall responses of aboveground stand characteristics to stand development, with young forests to the left and old forests to the right. Most of the underlying data is presented in more detail in paper I and II. Stand characteristics marked with * showed statistically significant differences between stand types according to ANOVA. Stand characteristics that showed a generally increasing or decreasing trend with time after clear-cutting are colored black or grey respectively.*

| Stand characteristic | Young (Clear-cut) | Old (Mature forest) |
|---|---|---|
| Tree biomass (Mg ha$^{-1}$)* |  | |
| Light transmission (%)* |  | |
| Total dwarf shrub biomass (Mg ha$^{-1}$)* |  | |
| *Vaccinium myrtillus* biomass (Mg ha$^{-1}$)* |  | |
| *Vaccinium vitis-idaea* biomass (Mg ha$^{-1}$) |  | |
| *Empetrum hermaphroditum* biomass (Mg ha$^{-1}$)* |  | |
| *Deschampsia flexuosa* biomass (Mg ha$^{-1}$)* |  | |
| Total dwarf shrub cover (%)* |  | |
| Total moss biomass (Mg ha$^{-1}$)* |  | |
| *Pleurozium schreberi* biomass (Mg ha$^{-1}$)* |  | |
| *Hylocomium splendens* biomass (Mg ha$^{-1}$) |  | |
| *Dicranum* spp biomass (Mg ha$^{-1}$) |  | |
| Total moss cover (%)* |  | |
| Moss depth (cm)* |  | |
| Litter depostition (g m$^{-2}$)* |  | |
| Number of tree stems per ha* |  | |

### 3.1.3 Moss layer

The moss layer was dominated by *P. schreberi* for all stand development stages, as is common in boreal forests (Frego and Carleton, 1995). The proportion of *P. schreberi* biomass to the total moss biomass varied between 56% (in the MF stage) and 73% (in the T1 stage) (paper I). The biomass of *P. schreberi* did not differ significantly between the MF stands and CC stands. Still, *P. schreberi* biomass was the lowest at the CC stands after which it quickly increased and doubled its biomass in the PCT and T1 stands, before decreasing in the MF stands (Table 1 and paper I). In contrast, *H. splendens* biomass was very low and made up between 6-18% of the total moss biomass. In the MF forest stands *H. splendens* biomass was highest, but it did not differ significantly from the other stages (Table 1 and paper I). Since clear-cutting is often reported to have negative effects on mosses, it was unexpected that no significant decreases were found for either of these moss species between before (i.e., MF stands) and after clear-cutting (i.e., CC stands), especially for *H. splendens*. These results were also surprising since it was expected that responses to stand development would differ between these two species with a relatively stronger negative response of *H. splendens* to clear-cutting. The non-responsiveness of *P. schreberi* to clear-cutting might at least partly be explained by it generally being better adapted to exposed locations (Gustafsson and Hallingbäck, 1988, Fenton and Bergeron, 2006) than *H. splendens*, which generally is more common in moist closed forests (Callaghan et al., 1978, Gustafsson and Hallingbäck, 1988), and is also reflected by differences in morphology and physiology (Proctor et al., 2007, Donath and Eckstein, 2010, Soudzilovskaia et al., 2011) and water holding capacity (Elumeeva et al., 2011, Michel et al., 2012) between the two species. In addition *P. schreberi* biomass already decreased between T1 and MF stands, which caused a relatively smaller impact of clear-cutting that was not enough to cause significant differences. The lack of response of *H. splendens* could partly be because when *H. splendens* occurred (especially in the CC stands), it was often found in patches that were more protected, e.g., behind stumps and below logs, where they would be less exposed and where higher moisture contents could be maintained (Schmalholz and Hylander, 2011). Overall moss depth was lowest in the CC stands and increased rapidly to the PCT stage; similar depths were found in the T1 and MF stages (Table 1, Figure 16).

Detailed studies on the biomass of understory vegetation are relatively uncommon in managed forests and most studies on field and moss layer vegetation report ground cover values rather than biomass values. The data from the chronosequence in this thesis (Figure 10) supports the notion that biomass and cover values are not interchangeable (Chiarucci et al., 1999) and

can show opposing trends (Muukkonen et al., 2006). Moss ground cover values along the clear-cut chronosequence were more in line with expected trends, with moss cover at the CC stands being lowest (49%) and increasing to around 70% in the PCT stands, where it stayed for the remainder of the stand development stages (paper I). As a consequence, clear-cutting significantly decreased moss cover compared to before logging values, in contrast to moss biomass which did not differ between before and after logging (Figure 10).

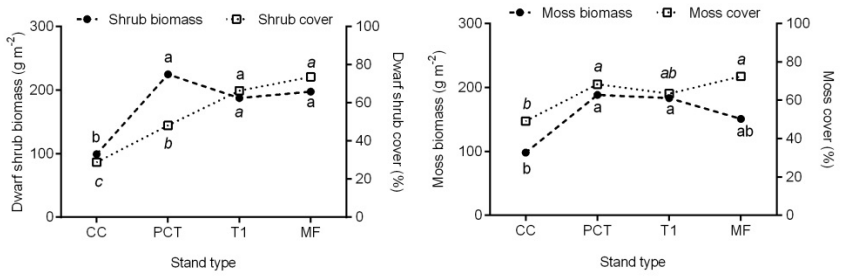


*Figure 10.* Responses of total dwarf shrub (left) and moss (right) biomass and cover for each of the chronosequence stages. Statistical significant differences between stand type are indicated by different letters, with non-italicized letters for biomass, and italicized letters for cover, according to ANOVA followed by Tukey's post-hoc test.

### 3.1.4 Soil

Despite large differences in the aboveground components of the stand (i.e., tree, shrub and moss biomass) among stand development stages, differences in the measured soil characteristics between stages were rare (Table 2). The bulk density of the soil in recent clear-cuts (CC) was significantly higher than in the T1 stands, possibly because of the recent use of heavy machinery (Greacen and Sands, 1980). In contrast, humus depth was highest in the MF stands, but was only significantly higher than in the PCT stands where the lowest humus depth was found (Table 2). The low humus depth in PCT stands was possibly caused by a combination of compaction, strongly reduced litter input after clear-cutting and sustained decomposition of the humus layer (Kreutzweiser et al., 2008). However, the differences in bulk density and humus depth did not result in a significant difference in humus mass between stand development stages (Table 2 and paper I). Soil nutrient values ($NH_4^+$, $NO_3^-$, $PO_4^{3-}$), element concentrations and total element pool (C, N, P) measured in the humus layer were not significantly different between stand types, except for significantly higher total C concentrations in the MF stands compared to all the other stand development stages (Table 2). Total element pool values per unit area were largely driven by variation in humus mass, and despite significant differences found for C concentration values across stand types; these did not translate to

significant differences in total C pool values. As such, clear-cutting followed by soil preparation did not exert a positive effect on total element pools or soil nutrients in the manner that has been claimed in some previous studies (Olsson et al., 1996, Rosén et al., 1996, Piirainen et al., 2007, Kreutzweiser et al., 2008). This might be because of key differences between those studies and this thesis, such as the types of measurements. For example, some of the studies have used measurements of leachates in stream water (Rosén et al., 1996) or mineral soil (Piirainen et al., 2007) to conclude that there is increased mineral N release after clear-cutting, while in this thesis nutrient values and elemental concentrations were measured in the humus layer. Additionally, at the time of soil measurements the CC stands were on average 5 years old, so initial transient increases in nutrient release may already have ended (Prescott et al., 2003, Kreutzweiser et al., 2008).

Table 2. *The overall responses of belowground stand characteristics to stand development, with young forests to the left and old forests to the right. Most of the underlying data is presented in more detail in paper I and II. Stand characteristics marked with * showed statistically significant differences between stand types according to ANOVA. Stand characteristics that showed a generally increasing or decreasing trend with time after clear-cutting are colored black or grey respectively.*

| Stand characteristic | Young (Clear-cut) | Old (Mature forest) |
|---|---|---|
| Humus bulk density (g $cm^{-3}$)* |  | |
| Humus mass (Mg $ha^{-1}$) |  | |
| Humus depth (cm)* |  | |
| $NH_4^+$ (mg $capsule^{-1}$) |  | |
| $NO_3^-$ (mg $capsule^{-1}$) |  | |
| $PO_4^{3-}$ (mg $capsule^{-1}$) |  | |
| N-concentration (%) |  | |
| P-concentration (%) |  | |
| C-concentration (%) * |  | |
| Total N (Mg $ha^{-1}$) |  | |
| Total P (Mg $ha^{-1}$) |  | |
| Total C (Mg $ha^{-1}$) |  | |

However, soil scarification in the CC stands was usually done more recently, ca. 3 years before soil measurements were taken. The disturbance and soil mixing effect of soil scarification is often suggested as one of the main reasons for elevated N mineralization and thus increased mineral N values in clear-cut stands (Frey et al., 2003, Piirainen et al., 2007). The positive effect of logging on N in northern Sweden has shown to generally last for a period of around six years (Olsson et al., 1996). Since measurements were taken within this time frame it was somewhat surprising that elevated mineral N was not detected in the humus layer.

It could be argued that despite what the soil measurements revealed, there are clear signs on increased mineral N as a result of clear-cutting, such as the high abundance of *D. flexuosa*. This grass is often considered to increase rapidly with increasing availability of mineral N (Nordin et al., 2006). However, it has also been shown that *D. flexuosa* abundance is highly controlled by light availability (Strengbom et al., 2004). *Deschampsia flexuosa* could therefore have positively responded to the greater light availability irrespective of whether mineral N had increased.

### 3.1.5 Variables driving differences between stand types

Inventories are time consuming, therefore it would be useful to be able to reduce the number of above- and belowground variables needed to properly describe the variation between different stand development stages. Those variables that explain a large proportion of the variation between stands might also be drivers of ecological processes in the understory. To accomplish this, a PCA was used to investigate which variables explained most of the variation between stands. Even though the variation explained by the first axis of the PCA analyses was driven by the trees, the differences in tree biomass only explained a relatively small part of the overall variation (22.7%) between stand development stages. This means that caution is needed when describing stand types and its possible impact on other ecological processes by only using the trees. The variation along the second axis (20.7%) of the PCA was driven more by the total soil C, nutrient pools and moss biomass. For the third axis (explaining 13.2% of the variation), the dwarf shrubs were the main driver. The second and third axes together explained a larger proportion of the variation than did the tree related factors from the first axis. Thus, in order to describe the variation between stands and assess the possible cause of the impact of stand type on processes in the forest understory, it is crucial to include measurements of the understory vegetation and soil characteristics. This confirms that the understory plays an important role in driving ecosystem

processes in these northern boreal forests (Nilsson and Wardle, 2005, Lindo et al., 2013).

## 3.2 Stand development effects on $N_2$-fixation rates

### 3.2.1 $N_2$-fixation capacity

Paper I showed that $N_2$-fixation capacity (i.e., $N_2$-fixation rates per unit moss mass) of both *P. schreberi* and *H. splendens* responded similarly to stand development stage, with the exception of the CC stands where $N_2$-fixation capacity of *H. splendens* was significantly higher than for *P. schreberi*. Both species showed their highest $N_2$-fixation capacity in the PCT stands, and much lower fixation values in the other development stages. Correlation analyses indicated that this increase of $N_2$-fixation capacity could have been caused by better habitat quality at the PCT stands as a result of increasing biomass of dwarf shrubs (especially *E. hermaphroditum*) and mosses. The presence of trees and dwarf shrubs would decrease wind exposure, allowing for a higher humidity on the forest floor (Økland et al., 2003), which would be further enhanced by the extensive presence of mosses that can be very effective in retaining moisture (Heijmans et al., 2004, Elumeeva et al., 2011). Enhanced levels of moisture in the moss layer, which is more typical in closed forests, have been shown to be beneficial for $N_2$-fixation by cyanobacteria (Gundale et al., 2009, Jackson et al., 2011). However, correlation analyses showed that increases in tree biomass had negative effects on $N_2$-fixation capacity, which may have resulted from low light transmission and decreasing temperatures on the forest floor (Gentili et al., 2005, Gundale et al., 2012a).

Another factor that might have stimulated $N_2$-fixation capacity in the PCT stands, and is often reported to be an important driver, is the lower availability of N (Zackrisson et al., 2004, DeLuca et al., 2008). When the availability of N is high, cyanobacteria may assimilate other N sources (Fay, 1992), and mosses may decrease the signaling compounds for $N_2$-fixing cyanobacteria (Bay et al., 2013). Although amounts of N and P in the soil did not differ significantly between stand development stages, the correlation analyses in Paper I suggested a negative relationship between $N_2$-fixation capacity and both $NH_4^+$ availability and total P pool size. The negative correlation between $N_2$-fixation capacity and $NH_4^+$ availability in the humus layer was in line with previous findings (Zackrisson et al., 2004, DeLuca et al., 2008). However, the negative correlation between $N_2$-fixation capacity and total P pool was unexpected, since $N_2$-fixation is an energy demanding process that requires P, meaning that increases in P availability often stimulate $N_2$-fixation (Vitousek et al., 2002,

Zackrisson et al., 2004). This result could have arisen because total P pool size might be a poor measure of actual P availability.

Additional factors such as the cyanobacteria community composition might also have contributed to the observed responses of $N_2$-fixation capacity specific to each moss species. It has been shown that certain cyanobacterial species are more efficient fixers than others (Ininbergs et al., 2011). It could also be that in the mosses of PCT stands more trace metals were available for the production of nitrogenase enzymes (Raven, 1988, Falkowski et al., 1998), which could stimulate $N_2$-fixation capacity.

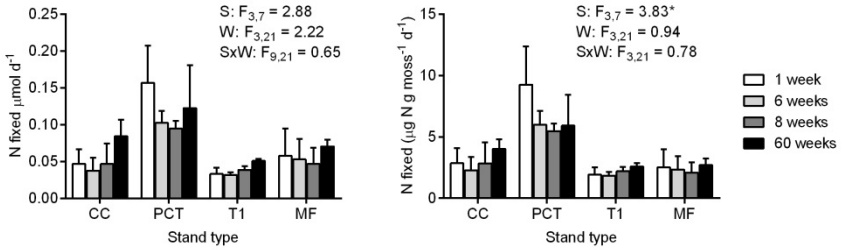


*Figure 11.* Effect of stand type and long term storage on *P. schreberi* associated $N_2$-fixation rates in µmol $d^{-1}$ (left) and N fixation rates per moss mass (right). Repeated measure ANOVA results are given in each sub-figure for stand type (S), number of weeks in storage (W), and their interaction. Bars show mean values + SE (n=3, except for CC n=2). * behind the F values indicate significant effects at the level $p < 0.1$

A long-term pilot study that involved the storage of *P. schreberi* samples from the clear-cut chronosequence in a climate chamber during 60 weeks suggested that the observed response pattern of $N_2$-fixation capacity to stand development was not entirely controlled by local conditions. The same samples were measured 1, 6, 8 and 60 weeks after collection in the field. This study instead showed that the pattern found across the chronosequence (with highest $N_2$-fixation in PCT stands) was maintained during 60 weeks of storage (Figure 11). This was despite the fact that mosses were kept in a climate chamber under constant conditions with optimal moisture at all times for all samples. It should be noted that the fixation values for N fixed per unit moss mass was only marginally significantly affected by type ($p < 0.1$), which may have been due to the relatively small number of replicates.

### 3.2.2 $N_2$-fixation at stand level

The total N input to the ecosystem, expressed as the amount of N fixed per unit area, increased tenfold between CC and PCT, and reached 1.0 kg $ha^{-1}$ $yr^{-1}$ (Figure 12). As *P. schreberi* was the dominant moss species, $N_2$-fixation rates

per unit area were largely influenced by the responses of this species. Total $N_2$-fixation rates per unit area were driven by both moss biomass and $N_2$-fixation capacity (per unit moss mass), showing that local conditions can influence the input of N into the ecosystem derived from feather moss associated $N_2$-fixation. The initial increase towards the peak values reached at PCT was a combination of rapidly increasing moss biomass and high $N_2$-fixation capacity. However, while moss biomass remained high after PCT, N fixed per unit area decreased likely due to lower $N_2$-fixation capacity. Overall, $N_2$-fixation rates per unit area were correlated with the same environmental factors as was $N_2$-fixation capacity.

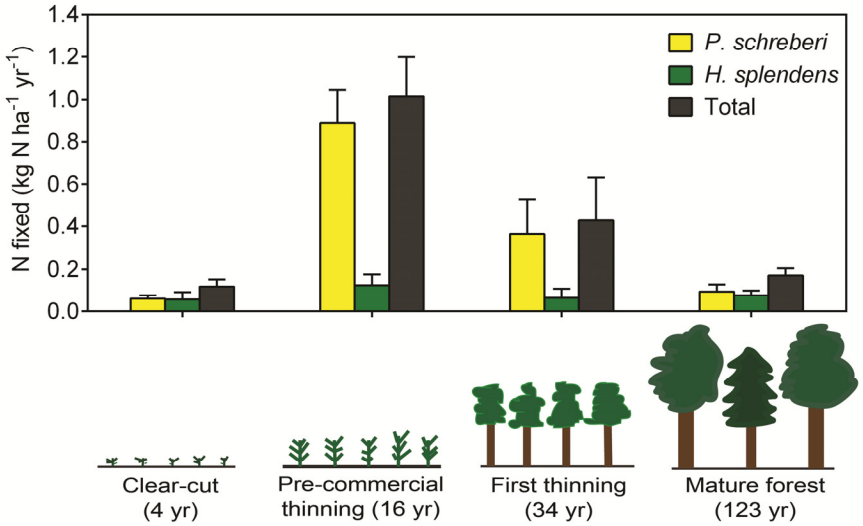


*Figure 12.* Amount of N fixed at each stand development stage for *P. schreberi* and *H. splendens* as well as their combined value, showing the annual input to the ecosystem per hectare for each developmental stage. (From graphical abstract paper I, published online in 2015 for Forest Ecology and Management, Elsevier)

As this is the first time to my knowledge that feather moss associated $N_2$-fixation has been investigated in managed forests, the only comparisons that can be made are with natural forests that have developed after fire (Lagerström et al., 2007, DeLuca et al., 2008, Gundale et al., 2012b, Lindo et al., 2013). However, those studies often span across much longer successional time scales, and investigations on stand ages as young as those featured in my work are mostly absent from the literature. Values similar to those found in the PCT stands have previously been reported for natural stands of approximately 200 years old (DeLuca et al., 2002, Zackrisson et al., 2004). This clearly indicates

that $N_2$-fixation rates cannot be predicted solely by stand age, but are also driven by local conditions at the specific stand level that are often influenced by management decisions. This also means that predictions about the potential contribution of $N_2$-fixation to the ecosystem N balance should take into account the differences between stand development stages, especially at the early stages of stand development.

### 3.2.3 Forest nitrogen balance

In N limited ecosystems such as the boreal forest, any addition of N could be of importance. An important question for understanding boreal forest N dynamics is therefore what the actual contribution of feather moss associated $N_2$-fixation is to the accrual of N during a forest stand rotation period. The removal of trees (often including logging residues) by clear-cutting results in removal of N from the stand (Palviainen and Finér, 2012, Merilä et al., 2014). This is also shown by the findings in paper I that after clear-cutting most N was stored in the humus layer. The amount of humus N decreased slightly, but not significantly, in the transition from the CC stands to the T1 stands. The total N content stored in aboveground biomass and the humus layer increased when stands reached the T1 stage, which was mainly due to the large increase in N stored in the trees. Overall, the total accrual of N between CC and MF was 520 kg $ha^{-1}$ at an average rate of 4.4 kg $ha^{-1}$ $yr^{-1}$. This increase of N to the ecosystem can come from several sources. One of those was explicitly investigated in this thesis, namely biological $N_2$-fixation associated with feather mosses. At the end of the rotation period of the stand $N_2$-fixation was estimated to have contributed to 9% of the total N accrual in the aboveground vegetation and humus layer at an average rate of *ca.* 0.4 kg $ha^{-1}$ $yr^{-1}$. The availability of this N for other vegetation is however still unknown, but it seems that the fixed N remains in the mosses for at least one year (Rousk et al., 2014). Interestingly, a large increase of N stored in the trees was observed between PCT and T1 stands. This increase followed the stage where peak $N_2$-fixation rates were measured, as such following a period with more N coming into the system. The pattern of increased N in the trees following peak $N_2$-fixation rates could indicate that the fixed N may become available to the trees at least during a time scale in the order of a couple of decades. However, more research is needed on the transition of fixed N by cyanobacteria to other vegetation.

Another source of N input to the ecosystem is atmospheric deposition, which is about 1-2 kg $ha^{-1}$ $yr^{-1}$ in northern Sweden (Phil-Karlsson et al., 2014). The contributions of feather moss associated $N_2$-fixation and atmospheric N deposition do however only account for *ca*. 45% of the total accrual. One likely

source of the remaining 55% of unexplained N input could be the mineral soil, which has shown to serve as a very large stock of N (Merilä et al., 2014) Within the scope of this thesis the contribution of $N_2$-fixation, *ca.* 50 kg $ha^{-1}$ during one rotation period, could be considered equal to the N content of twigs and branches (Palviainen et al., 2004). This would suggest that $N_2$-fixation could compensate for the N that is removed from the stand by harvest of slash (i.e., tops and branches) for bioenergy purposes.

## 3.3 Stand development effects on seedlings

As there is increasing interest in close-to-nature forest management that more closely resembles natural processes, natural regeneration is likely to become of greater importance (Puettmann et al., 2009). In order to use natural regeneration to its full potential in managed forests, it is important to get a better understanding of the survival and growth responses of seedlings to different developmental stages. In paper II, the response of seedlings of two different and commercially important tree species, with contrasting life history strategies, was examined along the clear-cut chronosequence. The reciprocal soil transplantation experiment between the stand development stages CC, PCT and T1 allowed for comparison of the impact of aboveground and belowground factors, independent of each other, on seedling performance. The results clearly showed that developmental stages can have both positive and negative effects on the performance of tree seedlings. The extent to which these effects were caused by stand type (i.e., above ground factors) versus by soil origin (i.e., belowground factors) differed between species, as well as between survival and biomass growth. These findings emphasize that causes of stand development effects on seedlings are variable and context dependent.

### 3.3.1 Aboveground factors

The results in paper II showed that seedling survival of both *P. sylvestris* and *P. abies* seedlings increased as stands got older. This effect seemed to correspond to the increased tree biomass and consequential canopy closure. For survival, the positive effects were likely due to facilitative effects of the canopy. With an increased canopy closure the humidity in the stand increases and temperatures will show less fluctuation (Groot and Carlson, 1996, Langvall and Örlander, 2001). Minimum temperatures on the clear-cut could be 3°C lower compared to closed forest, but even differences of up to 6 °C have been recorded (Groot and Carlson, 1996). This lower (night) temperature in the clear-cuts increases the risk that seedlings will get frost damage caused by frost events early during the growing season (Langvall and Örlander, 2001).

On the other hand, higher day temperatures on the young clear-cut also increase the risk of desiccation of the seedlings (Fleming et al., 1994, Greene et al., 1999). Most of the dead plants were still present in the cores and as such were not subject to predation, but rather desiccation or frost damage (personal observation). In those cases when the seedlings were no longer present in the cores predation by voles, or occasionally snails was most likely.

In contrast, biomass of seedlings of *P. sylvestris*, but not of *P. abies*, decreased when stands got older (Figure 13). This was likely caused by canopy closure which caused limitations in light availability for seedlings of *P. sylvestris* which is shade-intolerant, but less so for *P. abies* which is shade-tolerant (de Chantal et al., 2003a). Apart from possible negative effects of *V. myrtillus* biomass on *P. sylvestris* seedling growth (as suggested by the correlation analysis), no strong relationships were found between dwarf shrub biomass and seedling performance. This was despite shrub biomass increasing in a similar fashion as tree biomass with stand development. The negative effect of *V. myrtillus* was however most likely also an effect of competition for light, even though some belowground competition has been suspected in other work (Jäderlund et al., 1997, Wardle et al., 2008).

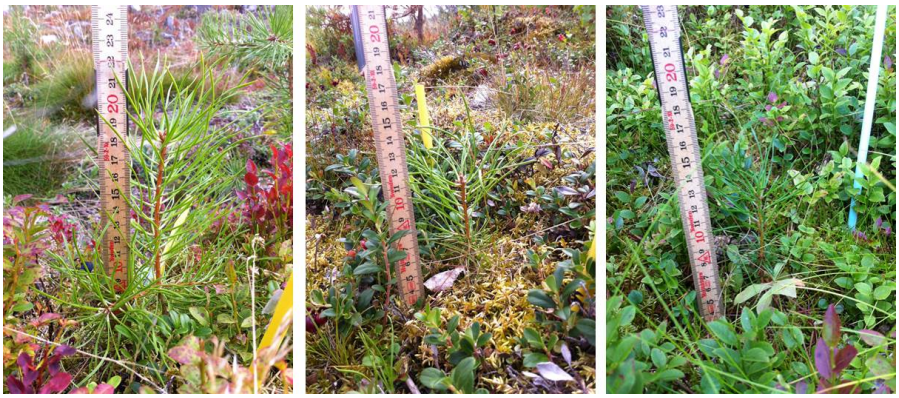

*Figure 13. Pinus sylvestris* seedlings growing in stands of different stand development stage in August 2014. Left: CC stand type (PCT soil origin), Middle: PCT stand type (PCT soil origin), and Right: T1 stand type (T1 soil origin).

The large variety of seedling responses to aboveground factors (paper II) shows the complexity of processes taking place at the forest floor. It appears however that both positive and negative aboveground effects of stand development on seedling performance were mainly driven by decreasing light transmission. It is most likely that the decline in light transmission was caused by canopy closure, but increasing biomass of *V. myrtillus* could also have been

a contributing factor. These findings showed that aboveground factors were of relatively greater importance for *P. sylvestris* than for *P. abies* seedling growth.

### 3.3.2 Belowground effects

While seedling biomass of *P. abies* responded little to differences in stand type (i.e., canopy effects), it showed strong responses to soil origin, with seedlings performing better in soil that originated from older stands (Figure 14). In contrast, seedlings of *P. sylvestris* showed no responses to soil origin. However, significant differences between stand development stages were not detected for the soil nutrient status (Table 2). Another possible explanation of different responses between soil origins might be caused by mycorrhiza. It has been shown that mycorrhizal fungal communities are different between clear-cuts and older stands (Jones et al., 2003). It is very likely that the ectomycorrhizal fungi that are associated with the tree species *P. sylvestris* and *P. abies* have become rarer at the clear-cuts when the trees were removed from the stand. When the seedlings were planted in soil originating from older stands, the mycorrhizae suited to these tree species were available and possibly symbiotic relationships were formed which gave the seedlings access to more nutrients. While *P. abies* seedling growth responded positively to the conditions in soils from older stands, seedling growth of *P. sylvestris* likely did not respond because of the overall stronger control of stand type (and thereby canopy effects). In contrast to *P. sylvestris,* this shows that for *P. abies* seedling growth soil conditions are of relatively more importance compared to aboveground factors.

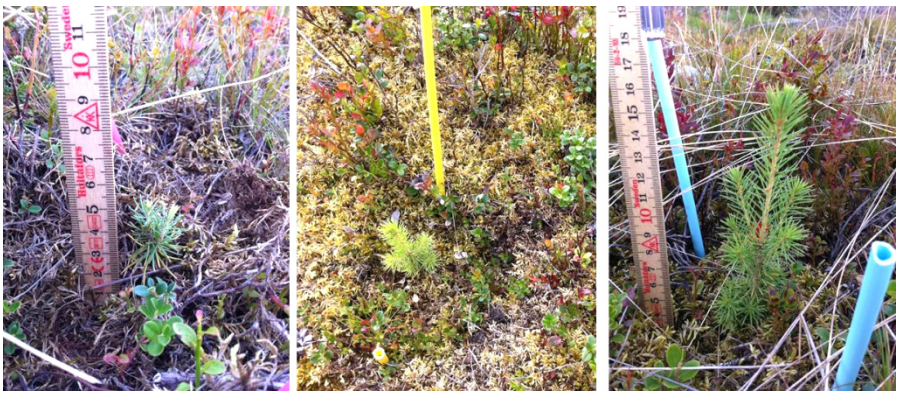

*Figure 14.* Three *P. abies* seedlings in the CC stand type in August 2014; in left: soil of CC origin, middle: soil of PCT origin and right: soil of T1 origin.

Additionally, it seemed that seedlings in general were most benefitted when grown in soil originating from older stands that was transplanted to stands with

high light availability (Figure 15). Seedlings generally grew best in soils from older stands when these were transplanted to the youngest CC stands. The results presented in paper II however only showed this to be significant when the statistical power was increased by analyzing data for both species in a single statistical analysis. Otherwise no interactive effects between stand type and soil origin were found for the individual species. Overall, the findings of paper II suggest that seedlings would initially benefit by the presence of a forest canopy to increase survival and perhaps improve their opportunities to initiate symbiotic relationships with mycorrhizal fungi. However, to maintain this advantage and have good growth, more open conditions are necessary for seedlings to develop further and ultimately become trees.

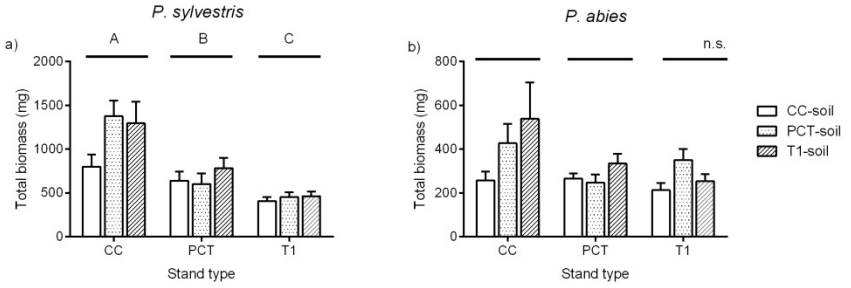


*Figure 15.* Total seedling biomass of *P. sylvestris* (left) and *P. abies* (right) seedlings grown in different stand types (x-axis) and soil of different origin (different bar patterns). ANOVA results for interactive effects between stand type and soil origin were not significant for the individual species (Paper II).

## 3.4 Effects of mosses on *Pinus sylvestris* seedling growth

The findings from the greenhouse experiment in paper III showed that effects of mosses on seedling growth differ depending on moss species identity and moss biomass. Effects of moss species on seedling growth were not significantly different from the moss free control. However, *P. schreberi* and *S. girgensohnii* generally stimulated *P. sylvestris* seedling growth, while *H. splendens* and *P. commune* generally repressed seedling growth. Apart from the moss species effects, the greenhouse experiment also showed that the effect of mosses on tree seedlings was influenced by moss biomass. When moss biomass was highest, seedling biomass declined compared to the other lower moss biomass classes. However, this decrease in seedling biomass was not significantly different from the moss free controls. The main cause of the negative effect of high moss biomass could likely be subscribed to high moss depth. Besides the increase in moss depth with higher moss biomass, moss

depth in the highest moss biomass class also differed between species due to their differences in morphology. The moss species *H. splendens* had the strongest negative effects, possibly because of its upright structure and broad-horizontal oriented leaves. In the greenhouse moss depths exceeding 70 mm strongly decreased seedling growth. In these cases, it appeared that the resources stored in the seeds were not sufficient for the seedlings to establish needles above the moss layer. In nature the maximum moss depth for seedlings to overcome might be a bit lower, due to differences in length of the growth season between seedlings and mosses. Since mosses continue to grow when seedlings already have stopped their aboveground growth, mosses can overgrow the crown of the seedlings later in the season (Hörnberg et al., 1997, Kellomäki, 1998). Thus in order to predict the effect of mosses on seedling regeneration it is important to consider the species composition of the moss layer.

Along the chronosequence *P. schreberi* was the most common moss species, and moss depth was on average 19, 32, 42, and 41 mm for the CC, PCT, T1 and MF stands respectively (Figure 16). Still, values over 70 mm were also recorded, and were most common in the T1 stands. Additionally, moss biomass levels in the proximity of the seedlings along the chronosequence were often around the 250 g $m^{-2}$ level, which is comparable to the 40% moss biomass class from the greenhouse experiment (Figure 16). As such, moss biomass was generally lower than 500 g $m^{-2}$, which was the maximum level tested in the greenhouse experiment. Neither of these levels showed negative effects of *P. schreberi* on seedling growth in the greenhouse experiment. As such, this could have contributed to the observation that moss biomass showed no negative effects on seedling biomass in the field. Along the chronosequence, mosses seemed instead to have a positive effect on seedlings. The positive effect of mosses on seedling growth along the chronosequence could have been due to co-variation with stand age, or due to maintenance of moisture in the top layer of the soil; it would need further studies though to confirm the latter. These findings contradict previous studies that have shown mosses to exert a strong negative effect on initial tree seedling establishment (Hörnberg et al., 1997, Zackrisson et al., 1997, Soudzilovskaia et al., 2011).

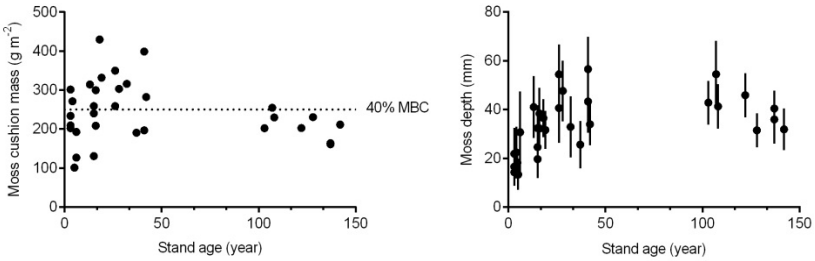


*Figure 16.* Average moss cushion mass (left) and average moss depth (± 95% CI) for each stand. 40% MBC refers to the 40% (250 g $m^{-2}$) moss biomass class used in the greenhouse experiment (paper III)

In addition to the impact of stand development on moss biomass and species composition and its subsequent effect on seedling growth shown in this thesis, a changing climate may also impact both moss biomass and seedling growth. Climate change predictions expect a reduction in summer precipitation frequency to occur, whereas the total amount of precipitation is expected to remain unchanged or increase (IPCC, 2007, May, 2008). My data from the greenhouse experiment showed that the strong reduction in precipitation frequency negatively influenced moss growth (paper III). It is possible that a reduced precipitation frequency could induce changes in moss species composition, and reduce the maximum moss biomass and depth that could be reached. The reduced precipitation frequency did not appear to affect *P. sylvestris* seedling growth. As such, it is not likely that the effect of mosses on seedling growth will change with future alterations of precipitation regime resulting from global climate change.

## 3.5 Implications

The work in this thesis has looked in detail at managed production forests from an ecological rather than a wood production perspective. Even though the development of the trees during stand development is well described (for example through growth development curves), much is still unknown about simultaneous changes in the understory vegetation or soil. The expected challenges for future forest management, that involve the realization of multiple ecosystem services, make it important to increase our knowledge about all layers in today's most common silvicultural system (i.e., the clear-cut system). It is my intention that the findings of this thesis could give forest managers and other researchers more insight into the functioning of stands that originate from clear-cutting. This could be used to evaluate possible benefits

and problems of these stands when one wants to change management systems, or improve current practices of forest management.

The findings in paper I highlighted the importance of preserving undisturbed patches of moss vegetation after clear-cutting for re-establishment of mosses. However, the findings in this thesis also showed the importance of the type of measurement (e.g., biomass vs cover) before drawing conclusions on the impact or recovery of disturbances on understory vegetation. For example, the cover of *V. myrtillus* recovered slower from clear-cutting than did the biomass of the same species.

Paper I also showed that relatively high $N_2$-fixation rates (i.e., 1.0 kg N $ha^{-1}$ $yr^{-1}$) that have been primarily associated with old natural forests (~200 years) can occur in young (~16 year old) production forests. If by management adjustment these $N_2$-fixation rates could be maintained during the whole of the rotation period, this would mean an input of 100-130 kg N $ha^{-1}$ to the ecosystem. This could lead to a substantial growth benefit during one rotation period. However, even at the rates achieved in today's conventional managed clear-cut initiated stands, $N_2$-fixation could contribute to about 9% of the total N increase in the aboveground vegetation and humus layer during a stand's rotation. This could indicate that $N_2$-fixation may potentially play an important though currently largely neglected role for the long-term N balance of stands in managed forests. This process might be one of the reasons why harvesting often does not appear to induce long-term deficiencies of N. Further research is required to determine the actual pathway of the N fixed by the cyanobacteria in order to ascertain when and how it might transfer to mosses and vascular plants. Nevertheless, all inputs of N should be of interest in these N limited ecosystems.

The study in paper II clearly showed that forest managers should consider maintaining (parts of) the canopy to increase seedling survival of both *P. sylvestris* and *P. abies* during the first couple of years after germination or planting, before the forest canopy is removed to benefit seedling growth. This would also contribute to maintain the positive effects of soils originating from older stands (in this case 16-34 years old) to further benefit early seedling growth of *P. abies* especially. Further research should focus on the identification of the actual source of the positive effect of soils originating from older soils on seedling growth. With regard to these species specific responses, the results from this thesis could also contribute to advance the transformation process from even-aged stands to multi-layered stands as is common in continuous cover forestry practices. This transformation process is commonly started from mature forests, however, there is an increasing interest

for instead transforming younger stands (e.g., T1 stands), partly because these are less sensitive to windthrow (Drössler et al., 2014).

The work on seedlings presented in this thesis focused on seedling growth, and did not consider seed germination. However, germination is a very important factor for tree establishment and can be influenced by understory vegetation. Moss layers have frequently been shown to have negative effects on seed germination, as they intercept the seeds, have a cooling effect on the soil (Hörnberg et al., 1997, Soudzilovskaia et al., 2011) and prevent the root of the newly germinated juveniles from reaching the soil (Zackrisson et al., 1997) where moisture availability is favorable and constant (Nilsson et al., 2002, Hille and den Ouden, 2004, Morgan, 2006). Nevertheless, it is likely that a certain percentage of the seedlings will successfully germinate, because of the large number of seeds deposited on the forest floor. It has also been shown that the type of seedbed favorable for germination may differ depending on the presence or absence of a canopy (Oleskog and Sahlén, 2000b, Oleskog and Sahlén, 2000a). Consequently, it is important to understand how mosses influence seedling growth both positively and negatively to allow managers to make well informed decisions.

The work in paper III showed that changes in moss species composition, biomass and climate could impact on moss depth and as such can affect *P. sylvestris* seedling growth. It also showed that the mosses only have negative effects on seedlings at high moss depth. This suggests that soil preparation is not necessary when moss layers are at those levels that were commonly found along the clear-cut chronosequence. These findings could have important implications for the understanding of forest regeneration in the future.

# 4 Conclusions and looking forward

## 4.1 Conclusions

Overall, clear-cutting has a large impact on the environment in forest stands. However, for some properties, but far from all, changes between stand development stages that occur during the long-term development after clear-cutting are larger than those that take place between pre- and post-logging. These aspects are often missing as most research has been done on short-term effects after clear-cutting, or have been done in natural forests along a much larger time scale that miss the rapid changes that occur during the first decades after stand initiation. The chronosequence that was established specifically for this thesis allowed for studies and comparisons of both short-term impact of clear-cutting and recovery over the following decades, a time-frame that is not so commonly considered in forest management and ecology. The general changes that occurred in the tree, understory, and soil during stand development following clear-cutting and the consequences for feather moss associated $N_2$-fixation and seedling performance are shown in Figure 17. Along the chronosequence the total biomass of mosses, field layer vegetation and trees, but not the nutrients stored in the humus layer, increased with stand development.

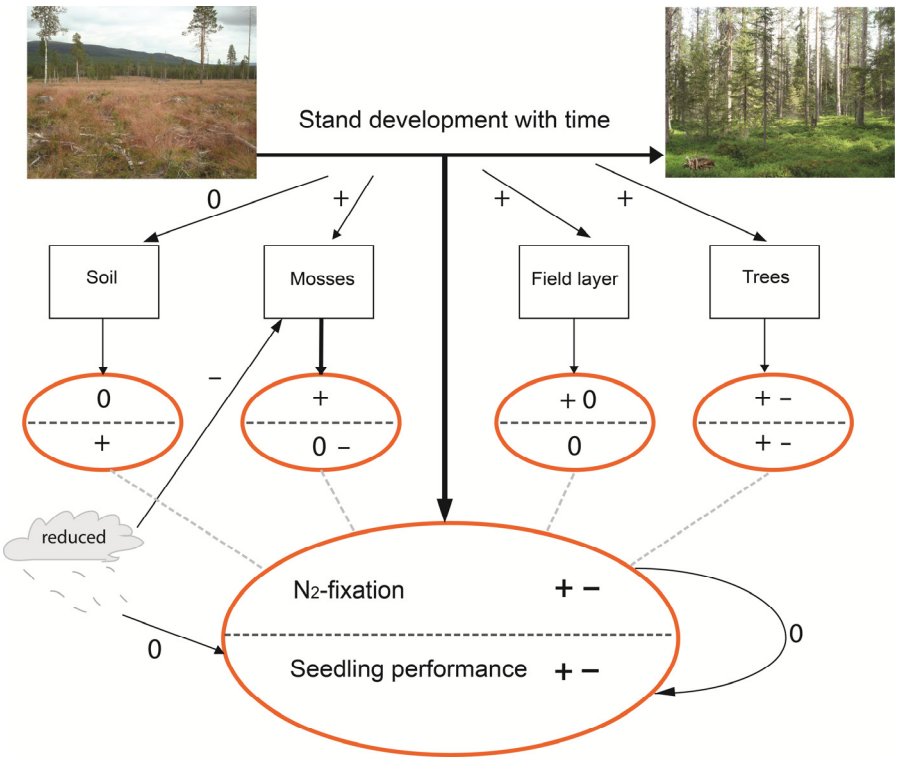


*Figure 17.* Summary of the effects of stand development on soil, understory vegetation and trees as well as the direct and indirect (through soil, understory vegetation and trees) effects of stand development on $N_2$-fixation and seedling performance. The four smaller ellipses refer to the effect of the above attribute on $N_2$-fixation (top half), and seedling performance (bottom half). The large ellipse refers to the direct effect of stand development on $N_2$-fixation (top half), and seedling performance (bottom half). The overall effects are marked with + (positive), 0 (neutral) or – (negative).

The work in paper I showed that after clear-cutting, $N_2$-fixation capacity first increased to a peak at around 16 years, after which it declined again to low levels. At its peak, $N_2$-fixation capacity was 6 and 3 times higher than in the other stand types for *P. schreberi* and *H. splendens*, respectively. The total N input per unit area due to $N_2$-fixation was also highest in the PCT stands and lowest in the recent clear-cut and mature stands. The increase of $N_2$-fixation per unit area between CC and PCT was probably driven by both $N_2$-fixation capacity and moss biomass, and could have been stimulated by lower $NH_4^+$ availability. The decrease after PCT however was mainly caused by decreasing $N_2$-fixation capacity, since moss biomass remained high. This showed that $N_2$-fixation rates did not follow a linear relation with stand age, and that local

conditions of the particular stand development stage are important for the magnitude of $N_2$-fixation rates.

Stand development affected seedling survival and seedling growth of *P. sylvestris* and *P. abies* differently. This was expressed by opposing direction or differences in the relative importance of above- and belowground factors. Seedling survival for both species (i.e., *P. sylvestris* and *P. abies*) increased with increasing stand age, and older stands likely facilitated the seedlings due to higher humidity and less temperature fluctuations and extremes. Seedling biomass of *P. sylvestris* was however negatively affected by the low light availability in the older stands, and the best growth performance of this species was therefore seen at the recent clear-cuts. *Picea abies* seedling performance was mostly influenced by soil origin, with soils originating from older stands being more beneficial for seedling growth. Measurements of the nutrient status of the soils along the chronosequence showed no differences between development stages. In the older stands, established mycorrhizal fungi communities provided by the presence of the established trees could have been well suited to the tree species used in the experiment and as such house better properties for seedling growth than soil from recent clear-cut stands. Seedlings grown in soils originating from older stands at the clear-cut showed better growth performance compared to seedlings grown in soil originating from the clear-cut itself. Since these differences were driven by soil properties, this seemed to confirm the suspected access to nutrients provided through the mycorrhizae. Still, this difference in growth between soil origins was only statistically significant when both species were analyzed together and at the recent clear-cut stands where light was not a limiting factor. However, there were more important differences in responses between the two seedling species, with *P. sylvestris* seedling growth controlled more by canopy conditions, and *P. abies* controlled more by soil properties.

The mesocosm set-up in the greenhouse showed that the impact of mosses varies depending on moss species and moss biomass. Negative effects of mosses on tree seedling growth were only found at high moss biomass, and specifically for *H. splendens*. The cause of these negative effects was mainly driven by high moss depth. Negative effects of mosses on seedlings were not found in the field along the chronosequence, possibly because the sites used were dominated by *P. schreberi* and not *H. splendens*, and because moss depth rarely exceeded 70 mm. In greenhouse conditions seedling performance was strongly inhibited when moss depth was more than 70 mm. In the future, declining precipitation frequency as a result of climate change would likely cause a decrease in moss growth and thereby moss biomass. In conclusion, this thesis has contributed to a better understanding of the interplay between

seedlings, mosses and $N_2$ fixing cyanobacteria in the actively managed production forests of northern Sweden.

## 4.2 Looking forward

The clear-cut system is like IKEA, in that you enter and follow a set path. As the customer is expected to follow a predictable path around the store, the trees planted on the clear-cut follow a predictable successional pathway. In this thesis, I have tried to explore the possibility of alternative pathways by improving the understanding of a variety of processes taking place on the forest floor. As for IKEA, one cannot deny that clear-cuts serve a clear purpose, as they efficiently produce wood. However, clear-cutting has created a more monotonous landscape, similar to most households that nowadays have a Billy bookshelf. Without an IKEA the customers are more likely to spread across a larger variety of stores. Most likely the absence of clear-cutting would also allow for a larger variety of silvicultural systems. Possibly, these systems would also be more influenced by local conditions, in a similar way that cupboards from Dalarna and Stockholm did not look the same many years ago.

While it is more natural for retail to adjust to customers' demands, the same adaptability cannot be seen in forestry. In my opinion important considerations for the future is to recognize that the clear-cutting system has proven to work very well, BUT it is not the only way. Nowadays, movements in society increasingly require a more diverse landscape and forests that provide a large variety of services other than wood production. The motivation can vary depending on the stakeholder, but it means in general that forests should provide a larger variety of ecosystem services than just wood production. This should be an incentive to improve an already working system. In other parts of Europe, things have already changed. Clear-cutting is done on a very limited scale or not at all. By learning from management practices in other countries and further studying the ecological processes and mechanisms specific for the managed forests in Sweden, the gained knowledge on natural processes could benefit a more diverse and potentially more successful forest management for a larger variety of stakeholders. Issues that deserve attention include: Where is the N fixed by cyanobacteria going? How and when does it become available for other vegetation? How can alternative silvicultural systems be implemented already today, before stands have reached their final age? Since old forests are more vulnerable to external disturbance such as storm damage, it could be sensible to start transitions already before this stage is reached. This is an opportunity for research to lead the way for future alternative forest management systems that also provide a variety of ecosystem services.

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***Propositions***

When I told my mother that I would write a PhD-thesis she told me she was looking forward to read my propositions as is tradition in Wageningen. When I told her that this is not common in Sweden she replied "How can you leave out the best part of a PhD-thesis???" So here they are:

- In the name of science you can wish for the Swedish sun to disappear
- Anything can happen during a PhD
- Whether field equipment is most suitable for science or berry picking can be a struggle.
- 2 is not so much, 4 is still not that much, 6 is still manageable, 8 is OK, but 2×4×6×8 becomes an overloaded pick-up, stuffed cooling room and many weeks of lab-work.
- Numbers of "knot" (midgets) and mosquitos are all relative to the summer of 2012.
- Even a PhD in natural sciences is mainly an education in English literature.
- The clear-cut system is like IKEA – *this thesis*
- drivers do not necessarily respond linearly – *this thesis*
- During a PhD you get to appreciate the power of positive feedback.
- It might take several years before noticing you're working in multiple languages.